MOLECULAR MARKER ANALYSIS OF QUANTITATIVE GENETIC TRAITS IN MAIZE

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By
Sarah Merriam Nourse

Dissertation Committee:

James L. Brewbaker, Chairperson
Henrik Albert
Rebecca Cann
Adelheid Kuehnle
Robert Paull

We certify that we have read this dissertation and that, in our opinion, it is satisfactory in scope and quality as a dissertation for the degree of Doctor of Philosophy in Horticulture

DISSERTATION COMMITTEE

James L. Brewbaker

Chairperson

Robert E. Paull

Adelheid R. Kuehnle

A. Albert

Robert Cain

DEDICATED TO MY GRANDPARENTS

Anna Frances Cooper
Milton Washington Cooper
Helen Valentine Nourse
Arthur Lesure Nourse

I ulu no ka lālā I ke kumu.
The branches grow because of the trunk

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I ha´aheo no ka lawai´a I ka lako I ka ´upena.

The fisherman may well be proud when well supplied with nets.

ABSTRACT

The three studies in this research incorporate molecular markers to analyze quantitative traits in maize. Molecular markers are used to identify quantitative trait loci (QTLs) and evaluate relationships and heterotic potential among selected supersweet inbred lines.

The first two studies incorporated a recombinant inbred line population, Set G, from the University of Hawaii derived from elite inbreds Hi31 (Hawaii/Iowa) and Ki14 (Thailand). The RIL population had been genotyped previously with 127 RFLP markers, creating a well-saturated linkage map (Ming *et al.*, 1997). Set G was used to study lime-induced chlorosis and photoperiod sensitivity in this research.

QTLs significantly associated with lime-induced chlorosis occurred on chromosomes 2, 3, 4, 5, 7 and 9. The QTL with largest effect was associated with RFLP locus *umc*26 on Chromosome 3, and explained 41% of the variation within the population.

QTLs significantly associated with photoperiod response were found on chromosomes 1,2, 5, 7, 9, and 10. The QTLs with largest effect were associated with RFLP locus php06005 at 62 CM on Chromosome 10, and explained from 9 to 39% of the variation within the population for the traits studied in both years, days to anthesis, days to silking, plant height, ear height, and leaf number below the ear.

The third study involved characterization of ten parental supersweet inbred lines from Hawaii, Thailand, Australia, and Iowa with 49 SSR markers. Genetic similarity coefficients were calculated for the inbreds based on the SSR analysis. A partial-diallel population was created with the characterized inbreds. Correlations (r) between GS

values with SCA estimates from diallel analysis were highest for days to silking and anthesis, plant and ear height, and ear length at (72%, 69%, 68%, 51%, and .49% respectively) and were all highly significant ($p > 0.01$). Tenderness ratings and GS values were also significantly correlated ($p = 0.05$). Sweetness and row number were not significantly correlated with GS values based on SSR analysis in this study. The GS values provided useful information about the diversity among the inbreds and insight into potential for hybrid performance for most of the traits evaluated in this study.

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LIST OF ABREVIATIONS

ANOV	analysis of variance
DTA	days to anthesis
DTS	days to silking
EH	ear height
EL	ear length
GCA	general combining ability
MAS	marker assisted selection
LN(be)	leaf number below the ear
QTL	quantitative trait loci
RAPD	random amplified polymorphic DNA
RFLP	restriction fragment length polymorphism
RIL	recombinant inbred line
RN	row number
SCA	specific combining ability
SSR	simple sequence repeat

PREFACE

This dissertation covers three studies combining traditional plant breeding objectives with molecular genetic tools to gain further insight into the genetic basis of the traits under study. Due to the variation in the topics, the first chapter of general introduction will cover the general concepts relative to all the studies with more detailed literature reviews within each chapter covering the specifics of each study.

In this research, molecular marker technology is incorporated to map quantitative trait loci (QTLs) associated with lime-induced chlorosis (Chapter 2) and traits associated with photoperiod sensitivity (Chapter 4) in a recombinant inbred line population of maize. SSR molecular markers are used to characterize 10 supersweet corn inbreds for analysis of genetic diversity and relationship to hybrid performance in a partial diallel series of crosses of the inbreds (Chapter 3).

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CHAPTER ONE

GENERAL INTRODUCTION

1.1 Maize Breeding and Quantitative Genetics

Plant breeding utilizes genetics and other disciplines such as pathology, entomology, physiology, statistics and biochemistry to improve the productivity, quality, and adaptability of crop plants. Once improved traits are identified, plant breeders work to incorporate the trait into new and existing varieties of that crop. Understanding of the genetic basis of any trait helps the plant breeder to predict the best and quickest method for incorporation. The ultimate success of a plant breeding program can be measured by the amount of time necessary for the incorporation of new traits into the crop of interest (Fehr, 1987).

Most traits of importance to plant breeders are quantitative genetic traits. These include stress tolerances, disease and insect resistances, and desirable morphological characteristics affecting quality, productivity and marketability of the crop. These quantitative traits require quantification of the variation in order to classify the phenotype, are not usually controlled by single genes and are generally complex in inheritance (Helentjaris, 1989). Despite the complexity, it has been well established that quantitative traits also behave in accordance with Mendelian principles (Comstock 1978). Genomic regions associated with quantitative trait inheritance are referred to as quantitative trait loci, QTLs.

1.2 Populations

Two types of populations that have been utilized in this research are recombinant inbred lines and the diallel series of crosses. In order to study the genetic basis of expression of a trait we must have germplasm that segregates for the trait or is variable for expression of the trait. Crossing schemes and various population types have been incorporated in plant breeding and genetic studies to achieve better understanding of the genetic basis of trait expression.

1.2.1 Recombinant inbred lines, RILs

Recombinant inbred lines (RILs) are an excellent tool for identifying QTLs. RILs can be derived rapidly in maize by selfing the progeny of an F1 hybrid via single seed descent for six or more generations (Burr and Burr, 1991; Moon and Brewbaker, 1999) resulting in a population of inbred lines that are ultimately fixed for different combinations of linked blocks of parental alleles. These inbred lines can be easily maintained and represent a "immortalized" population that is suitable for repeated experimentation under diverse stresses and environments.

The selection of parental lines that are diverse for the expression of many traits, increases the utility of the population studies. Moon developed 9 RIL populations based on crosses of temperate by tropical germplasm at the University of Hawaii including Set G utilized in two studies in this dissertation (Moon, 1995). Additionally, three more RIL populations have been developed since 1995. These RIL populations have been studied for many traits around the world (Moon and Brewbaker, 1999).

Two of the University of Hawaii RIL populations Set G and Set I have been genotyped with molecular markers producing well-saturated linkage maps (Ming, 1995) (Lu, 1999) which have allowed for the identification of QTLs in Set G associated with maize mosaic virus (Ming *et al.*, 1997), maize streak virus (Kyetere *et al.*, 1995), Stewart's wilt (Ming *et al.*, 1999), lime-induced chlorosis tolerance (Nourse *et al.*, 1999), and photoperiod response. QTLs have been identified in Set I associated with common rust (Lu, 1999), corn head smut (Lu, 1999), fall army worm and sugarcane borer resistance (Lu, 1999). The diversity and number of QTLs identified with these two RIL populations demonstrates the vast potential for genetic analysis that these populations provide.

1.2.2 Diallel Crosses

A diallel series of crosses provides the plant breeder and geneticist with a unique look at the performance of the parental lines in different cross combinations and insight into the gene action controlling expression of the traits under study. A full diallel cross is comprised of a series all possible combinations of single crosses among 'n' parents, the number of subsequent F1 crosses represented by n^2. A partial diallel would combine 'n' parents in half of the possible combinations to result in [n(n-1)/2] F1 crosses. A nine parent partial diallel would have 36 resultant F1 crosses.

There are two well-established models for diallel analysis, Griffing (1956) and Gardner and Eberhart (1966), used to estimate genetic effects from a diallel series of crosses. Basic assumptions of these models include diploid inheritance, two alleles per

locus, and no epistasis. These assumptions limit extrapolation from the results and have invited criticism of this type of analysis (Baker, 1978).

Diallel analysis, however, with either model provides a method for estimating the genetic effects of the diallel cross by partitioning the effects into general and specific combining abilities. The general combining ability, GCA, is the effect that the parent has on the progeny cross for the trait measured. GCA is the expression of the additive genetic effects, those that can be most easily influenced by selection and where the plant breeder can make rapid gain through selection. Specific combining ability, SCA, represents the departure from additive effects and is represented by a parent's superior or inferior performance in a cross compared to expectations based on parental performance. SCA includes any non-additive effects like dominance, epistasis, or multiplicative gene action (Sprague and Tatum, 1942).

Diallel analysis utilizes the ANOV of a randomized complete block design or other design and subdivides the hybrid mean square into the components of GCA and SCA. The basic model is represented by:

$$Y_{ij} = u + g_i + g_j + s_{ij} + e$$

Where Y_{ij} is any hybrid mean, u is the mean effect for all hybrids, g_i and g_j are GCA effects of the i and j parents, s_{ij} is the SCA effect for a particular hybrid, and e the error component. Heritabilities of the traits under study can also be estimated based on mean squares generated from the ANOV for combining abilities (Brewbaker, 1994).

1. 3 Genetic Markers

Quantitative traits have long been associated with genetic markers (Sax 1923). The incorporation of genetic markers to aid in the selection of quantitative traits has been given considerable attention in plant breeding research (Van Hintum, 1994). These genetic markers were initially morphological, but there are many negative attributes of such markers. They are limited in number, usually not desirable in the final product and cumbersome to work with.

Isozyme markers were the first widely used biochemical marker. Isozymes are enzymes that share a common substrate specificity, but differ in electrophoretic mobility. They are revealed after tissue extracts are subjected to electrophoresis and stained in solutions containing enzyme specific components (Doebley and Wendel 1989). The data from the isozyme banding patterns can be translated to a single or multilocus genotype for each individual analyzed. Many isozyme banding patterns have been linked to commercial traits of interest and are incorporated in plant breeding for major crops like tomato. Despite their advantages of being technically simple and relatively inexpensive, they represent a limited number of markers due to a small number of enzyme systems.

1.4 Molecular markers

Molecular markers like RFLPs, RAPDs, and SSRs reflect chemical variation at a particular site of DNA (Stam, 1993). Advantages of molecular markers include a high level of allelic variation, phenotypically neutral behavior, more precise evaluation procedure without epistatic and environmental effects, a potentially unlimited resource,

the potential to replace time-consuming and complicated screening procedures, and the ability to evaluate plant material at a seedling stage (Tanksley *et al.*, 1989).

1.4.1 Restriction Fragment Length Polymorphisms (RFLP)

RFLPs were first described in 1980 (Botstein *et al.*, 1980) and are frequently employed to detect genetic polymorphism. Polymorphisms occur as a result of changes in restriction enzyme sites due to point mutations, duplications, deficiencies, or genetic rearrangements (Burr and Burr, 1991). Hybridization with radio-labeled probes containing sequence homologous regions of chromosomal fragments permits identification of specific loci (Devey and Hart, 1989). These makers are abundant, can be mapped to specific loci on chromosomes and are highly polymorphic in maize. Although they are technically relatively difficult, they remain the backbone of molecular marker analysis in maize.

1.4.2 Random amplified polymorphic DNA (RAPD)

RAPD markers provide a DNA polymorphism assay based on the amplification of random DNA segments with single primers of arbitrary nucleotide sequence (Williams *et al.*, 1990). Techniques are based on the use of polymerase chain reaction, PCR. PCR allows direct amplification of defined DNA segments from genomic DNA, and permits the analysis of template DNA of virtually any organism (Saiki *et al.*, 1988). The polymorphisms between individuals result from sequence differences in one or both of the primer sites and are visible as the presence or absence of a particular RAPD band. RAPDs have the advantage of being technically simple, quick to perform, require small

amounts of DNA, and in most cases involve no radioactivity. RAPD markers behave as dominant genetic markers which limits their utility in genetic mapping (Bell and Ecker, 1994) because of the inability to score heterozygotes.

1.4.3 Microsatellite based PCR markers

Microsatellites or simple sequence repeats, SSR, are markers first described by Litt and Luty (1989), Tautz (1989), and Weber and May (1989). They soon became a molecular marker system of choice (Ellegren, 1993). Microsatellite based PCR makers are based on simple sequences of DNA which consist of stretches of monotonously repeated short nucleotide motifs (Tautz, 1989). These microsatellite motifs are generally characterized by repetitive di-, tri-, and tetra-nucleotide sequences that occur abundantly and at random throughout most eukaryotic genomes. Specific microsatellite islets contained within a stretch of DNA can be individually amplified via PCR using a pair of flanking oligonucleotides that point inwards towards the bracketed microsatellite motif (Beckman and Soller, 1990). The length of the amplified region will normally be within a range of 50-300 bp which allows for resolution of the amplification product on standard sequencing gel as well as on agarose gels (Zhao and Kochert, 1993).

Microsatellite sequences can be isolated directly from the genome of the species under consideration or found through the screening of databases for species with available sequence databases. Several investigations in plants have demonstrated that SSRs are informative, randomly distributed throughout the genome (Wang *et al.*, 1994), and show codominant Mendelian inheritance (Devos *et al.*, 1995), greatly facilitating the analysis of the patterns obtained in population studies. The main drawback of SSRs is

the effort required to find them (Queller *et al.*, 1993). Another problem is the frequent occurrence of non-related amplification products, due to chance homologies of the primer at other sites (Tautz, 1989).

1.5 QTL Mapping

1.5.1 Linkage map construction

Paterson (1988) first described molecular marker linkage maps in plants. These linkage maps have far surpassed traditional morphological or isozyme-based linkage maps in their degree of resolution (Dudley, 1993). Molecular marker analysis consequently creates the ability to assay all regions of the genome, providing accurate estimates of the phenotypic effects and genetic position derived from interval analysis. Molecular marker analysis ultimately allows the resolution of quantitative traits into discrete genetic regions known as quantitative trait loci (QTLs) (Lander and Botstein, 1988). Molecular linkage maps have been developed for a number of major crop species including maize (Coe *et al.*, 1995). The development of these well-saturated molecular marker linkage maps has verified and vastly expanded the knowledge of gene and QTL location and expression in maize and other crops.

1.5.2 Interval Mapping

The basic premise for mapping QTLs is that the molecular markers and the QTL are linked. The interval mapping method was described by Lander and Botstein (1989). Interval mapping measures the effect of the genomic regions between two molecular markers to identify regions associated with the phenotypic expression of the trait. The

computer program MAPMAKER/QTL (Lincoln *et al.*, 1993) was developed to identify QTLs with the interval mapping technique. MAPMAKER/QTL calculates LOD scores, the Log_{10} of the odds ratio which represents the probability that the data arises from a QTL over the probability that the data arises by chance alone. The threshold for LOD values is set based on the parameters of the experimental population and the molecular marker linkage map. Generally, a LOD greater than 3 (P=.001) is considered to be a significant indication of a putative QTL. In this work I utilized MAPMAKER/QTL to identify intervals containing putative QTLs and then use single factor analysis of variance to confirm the importance of the markers in that interval and verify QTL location.

1.5.3 Single factor analysis of variance

The single factor analysis of variance is essentially a linear regression of the phenotypic data set with the genotypic (molecular marker) data by way of a one-way analysis of variance (Lander and Botstein, 1988). I utilized the PROC-GLM procedure of SAS (SAS, 1989) to calculate the single factor analysis of variance in these studies. The R^2 values generated represent the percentage of variation that a single molecular marker explains in the population. Any marker that has a significant F value based on this analysis can be considered to be associated with the expression of the QTL. This analysis can further pinpoint the effect of a single marker within an interval identified by MAPMAKER/QTL, confirming the location of the QTL and giving another measure of its importance to the expression of the trait.

1.6 Applications of molecular marker analysis

1.6.1 Heterotic Grouping

A primary objective of maize breeders is to define heterotic groups to maximize exploitation of heterosis. Much remains to be known about the genetic, biochemical, and physiological basis of heterosis, but it is often considered to be the rational for hybrid maize breeding (Melchinger *et al.*, 1990a). Molecular markers like RFLPs offer a relatively new method of characterizing germplasm with improved resolution and allowing better definition of the resources available (Evola *et al.*, 1986). The premise is that diversity on the molecular level should provide a means of fingerprinting germplasm, determining relatedness of germplasm (genetic distance), classifying germplasm into heterotic groups, and ultimately helping to predict hybrid performance (Bernardo, 1992; Godshalk *et al.*, 1990; Livini *et al.*, 1992; Melchinger *et al.*, 1990b).

Studies utilizing molecular marker-based estimates of genetic distance as a predictor of hybrid performance have given mixed results, some positive (Lee et al. 1989, Melchinger 1992) and some with low correlations (Godshalk *et al.*, 1990; Melchinger *et al.*, 1990b). However, Marsan et al. (1998) studied a diallel population (parents genotyped with RFLPs and AFLPs) and found when genetic distances based on the molecular marker analysis were partitioned into general and specific genetic distances, as with trait evaluations in a diallel analysis, the predictive value was enhanced (Marsan *et al.*, 1998). An overall objective of this kind of evaluation would be to decrease the time necessary for cultivar development, a major objective of any breeding program.

1.6.2 Marker assisted selection

Marker-assisted selection, MAS, has been proposed by a number of authors (Lee, 1995; Tanksley, 1993) to minimize expenditures of resources and time associated with field screening. In backcross breeding programs in particular it has been shown that MAS can reduce linkage drag by selecting for only the alleles desired from the donor genome (Van Berloo and Stam, 1998).

CHAPTER TWO

IDENTIFICATION OF QUANTITATIVE TRAIT LOCI ASSOCIATED WITH LIME-INDUCED CHLOROSIS

2.1 Abstract

Lime-induced chlorosis in maize is commonly associated with iron deficiency in high pH soils. Calcareous soils of this type are relatively common in the lower rainfall areas of the tropics and limit maize production especially in Mexico and the Middle East. Scientists at the International Maize and Wheat Improvement Center (CIMMYT) in Mexico have a breeding and physiological program for improving tolerance to such soils at their Tlaltizapán station in Morelos, Mexico. In 1997 we evaluated 100 recombinant inbred lines (RILs) derived from elite inbreds Hi31 (Hawaii/Iowa) and Ki14 (Thailand) in four replicated trials on iron-deficient soils at the Tlaltizapán station. Leaf chlorosis was evaluated by visual ratings and with a Minolta SPAD-502 Chlorophyll Meter during the first 8 weeks of growth. The parents differed significantly in all trials with Hi31 susceptible and Ki14 highly tolerant. RIL inbreds varied widely between the parental extremes, with approximately one fourth in each of the parental classes. The RIL population had been genotyped previously with 127 RFLP markers, creating a well saturated linkage map (Ming *et al.*, 1997). Putative quantitative trait loci (QTLs) were analyzed through single-factor ANOVA and with MAPMAKER\QTL interval mapping. QTLs significantly associated with lime-induced chlorosis occurred on chromosomes 2, 3, 4, 5, 7 and 9. The QTL with largest effect was associated with RFLP locus *umc*26 on Chromosome 3, and explained 41% of the variation within the population. Two intervals on the long arm of chromosome 3 also gave the highest observed LOD scores for

chlorosis. High LOD scores were also recorded for the interval between *umc*156 and *umc*200 on chromosome 4. These QTLs are believed to be good candidates for marker-assisted selection to overcome problems associated with cultivation of maize on iron-deficient soils.

2.2. Introduction

Iron deficiency is the primary factor in lime-induced chlorosis of plants grown on calcareous soils. The chlorosis is not due to free lime *per se* but to the high pH and lowered solubility of ferrous iron (Olson and Lucas, 1967).Calcareous soils account for approximately one third of the earth's surface (Brown and Jolley, 1989), and limit the expansion of agriculture in lower rainfall areas especially in Mexico and the Middle East. These soils are strongly buffered near pH 8, where iron solubility is at its lowest (Lindsay, 1995). Optimal plant growth requires 10^{-9} to 10^{-4} M iron, whereas total soluble iron in calcareous soils maximizes at 10^{-10} M (Briat and Lobreaux, 1997; Guerinot and Yi, 1994).

Iron is an important element because of its participation in most of the basic redox reactions in the production and consumption of oxygen, and its involvement in many vital enzymatic reactions (Briat and Lobreaux, 1997). The symptoms of lime-induced chlorosis in maize (*Zea mays L.*) range from mild interveinal striping to a bleached appearance of the entire leaf, accompanied by poor plant growth and often by death (Choi, 1996). A maize breeding effort directed towards tolerance to lime-induced chlorosis was initiated at CIMMYT in 1985, following the observation of a high degree of tolerance in the maize landrace, Puebla 209 (Edmeades, *pers.comm.*). The soil at the

Tlaltizapán Research Station of CIMMYT in Morelos, Mexico, is highly calcareous and low in native iron. High levels of calcium salts and of bicarbonates occur in the irrigation water, enhancing conditions for lime-induced chlorosis.

Two monogenic recessive mutants in maize that develop interveinal chlorosis are the yellow-stripe loci, *ys1* and *ys3*. Mutant *ys1* is located on chromosome 5 and affects the uptake of phytosiderophores (Wiren *et al.*, 1994). Mutant *ys3* is on chromosome 3 and is considered to affect the capacity to secrete phytosiderophores into the rhizosphere (Basso *et al.*, 1994). Phytosiderophores have the capacity to chelate ferric iron, resulting in ferric siderophores which are recognized and transported through the plasma membrane (Marschner and Romheld, 1995; Romheld, 1987). Maize and many other grasses incorporate this strategy under iron limiting conditions.

Champoux et al. (1988) studied maize *wx1*- and *su1*-translocation stocks to identify chromosomal regions associated with lime-induced chlorosis in Nebraska. Inbred line Purple 3036-1 of unknown origin was the source of tolerance. Regions on the long arms of Chromosomes 3 and 8 were identified as sites of major genes for tolerance to lime-induced chlorosis. Regions on Chromosomes 2, 4, 6 and 10 were also shown to affect tolerance to a lesser degree.

Recombinant inbred lines (RILs) provide the geneticist with an excellent tool for identifying quantitative trait loci (QTLs). They can be derived rapidly in maize by selfing the progeny of an F1 hybrid via single seed descent six or more generations (Burr and Burr, 1991; Moon and Brewbaker, 1999). RIL populations are fixed for different combinations of linked blocks of parental alleles, and are suitable for repeated experimentation under diverse stresses and environments for genetic analyses. The

University of Hawaii has developed a series of eleven RIL populations based largely on temperate by tropical crosses (Moon and Brewbaker, 1995), including the RIL population Set G used in this study.

Two major QTLs have been mapped in Set G prior to this study. They are resistance to Maize Mosaic Virus (MMV) on the long arm of chromosome 3 and resistance to Stewart's wilt (*Erwinia stewartii* Smith) on the short arm of chromosome 1 (Ming *et al.*, 1999; Ming *et al.*, 1997). Many other QTLs segregate in this germplasm, as it represents two very different parents—one a temperate dent and the other a tropical flint. The elucidation of QTLs, and particularly QTLs with large effect, provides information that can be directly applicable to maize breeding. Marker-assisted selection for QTLs can ultimately increase the efficiency of selecting for traits such as abiotic stress, disease and insect tolerance (Lande, 1992).

This study was conducted to evaluate lime-induced chlorosis segregating among RILs of Set G with the objective of identifying QTLs associated with tolerance to this stress.

2.3. Materials and Methods

2.3.1. Plant Material

The primary maize population in these studies was Hawaii Set G, a recombinant inbred line (RIL) population based on the hybrid of inbreds Hi31 and Ki14. The temperate dent line Hi31 was derived from the inbred lines B68 and RB14A out of 'Iowa Stiff Stalk Synthetic', and the tropical flint line Ki14 was derived from 'Suwan 1', a Thai flint population (Brewbaker, 1997). The F1 plants were self-pollinated and carried through to F7 via single seed descent, resulting in 129 RILs (Moon and Brewbaker,

1999). This population was previously genotyped with 127 RFLP probes, and a well saturated linkage map was developed of total length 1625 cM, with an average interval of 15.8 cM between markers (Ming *et al.*, 1997). Two major QTLs have been mapped in Set G prior to this study, resistance to Maize Mosaic Virus (MMV) on the long arm of Chromosome 3 and resistance to *Stewart's* wilt (*Erwinia stewartii* Smith) on the short arm of Chromosome 1 (Ming *et al.*, 1999; Ming *et al.*, 1997). Several other mapping studies are currently on-going with this germplasm. Nine additional inbreds used as parents in other University of Hawaii RIL populations were also planted for observation in these experiments. These represented a wide variety of temperate and tropical germplasm, and were inbreds B73, DB544, Fla2Bt73, Hi34, Mo17, Nariño 330, Tx601, TZi4 and TZi17 (Moon and Brewbaker, 1999). Ten or more sub-lines of each parent were bred and evaluated as sister lines to provide greater accuracy in the assessment of parental line means and variances.

2.3.2. Field Evaluation

Field evaluations were conducted at the experimental station of the International Maize and Wheat Improvement Center (CIMMYT) near Tlaltizipán, Mexico, which is located in a mid-altitude tropical climate at 940 m altitude in the central mountains of Mexico (18°41'N, 99°08'W). The soil at Tlaltizapán is classified as clay Pellustert (Vertisol), and is highly calcareous and low in native iron. High levels of calcium salts and bicarbonates occur in the irrigation water, enhancing lime-induced chlorosis.

One hundred RILs of Set G, ten sub-lines of parents Hi31 and Ki14, the nine supplementary inbred lines, and one CIMMYT inbred line were evaluated during the dry

season of 1997 in a 13x10 (0,1) alpha lattice design (Patterson and Williams, 1976) with two replicates. Plot size was one row of 5.25m length. Seeds were over-sown on 17 December 1996 and seedlings were thinned to a density of 6.67 plants m^{-2} after establishment. Between- and within-row distances were 0.75 and 0.2 m, respectively. Water was furrow-irrigated as needed to avoid water-limited growth. This experiment was planted as a part of a photoperiod sensitivity experiment on land with moderate iron availability, and is referred to as EXP1.

A third replicate was planted in a separate field at the Tlaltizipán station with more abundant iron present in the upper soil horizons, and all lines were sib-increased there. Sufficient healthy seed was obtained from 91 RILs and from the parental sub-lines and the supplementary inbred lines. Following the 1997 winter season evaluations, three trials were planned for the 1997 wet summer season. Using the fresh seed, the 91 RILs, the ten sub-lines of each of both parents, and the nine supplementary inbred lines were sown in a 12x10 (0,1) alpha-lattice design with two replicates per experiment. The plant material was evaluated in three spatially separated fields with different levels of iron availability (Table 2.1). These are referred to as fields with low (EXP4), medium (EXP2) and high (EXP3) iron availability, respectively, and were sown on 29 May, 1 July and 11 June. One-row plots of 2m length were used in EXP4, and one-row plots of 2.5m length were used in the EXP2 and EXP3. Management practices were as described for the 1997 dry season.

Table 2.1. Soil properties of the three experimental fields near Tlaltizapán, Mexico, that differed in iron availability and that were used during the 1997 wet season.

Experiment	Fe Availability	Depth	pH	Ca ppm	Fe ppm	Soluble N(%)	Organic Matter (%)
EXP2	Medium	0-30	7.5	8738	5.1	0.124	2.40
		30-60	7.5	8921	5.6	0.094	1.96
		60-90	7.4	9227	6.5	0.088	1.75
		average	7.5	8962	5.7	0.102	2.04
EXP3	low	0-30	7.2	5799	3.5	0.128	2.45
		30-60	7.3	6627	3.4	0.090	1.86
		60-90	7.4	7296	4.5	0.084	1.71
		average	7.3	6574	3.8	0.101	2.01
EXP4	high	0-30	7.4	7311	8.6	0.158	3.20
		30-60	7.4	7205	10.0	0.118	2.34
		60-90	7.5	6654	6.5	0.063	1.16
		average	7.4	7057	8.4	0.113	2.23

Fields with low, medium and high iron availability were characterized by iron concentrations of 3.8, 5.7 and 8.4 ppm, respectively, between 0 and 90 cm depth (Table 2.1). Calcium concentrations of these fields were 6574, 8962 and 7057 ppm, respectively. Soluble nitrogen and organic matter contents, and pH values did not differ substantially among the three fields.

Visual ratings of chlorosis were taken on a scale of 1 to 9 at four weeks after planting in EXP1, 1 being healthy and 9 corresponding to 100% chlorotic. The ratings were repeated at six weeks after planting on a 1 to 5 scale, 1 being healthy and 5 completely chlorotic. Visual ratings were taken on this scale 5, 3, and 3 times in EXP2, EXP3, and EXP4, respectively, during the first eight weeks of growth.

A measure related to leaf chlorophyll concentration was determined in all experiments with a Minolta SPAD-502 chlorophyll meter (hereafter 'SPAD') from expanded leaves. During the first eight weeks of growth SPAD readings were taken 1, 4, 1, and 2 times in EXP1, EXP2, EXP3 and EXP4, respectively. This portable device can be used to measure green color nondestructively in plant tissue, and readings normally correlate well with chlorophyll concentrations (Yadava, 1986). The SPAD meter has also been used to provide an unbiased measure of the severity of leaf chlorosis associated with iron deficiency in soybean (Peryea and Kammereck, 1997).

2.3.3. Statistical Analysis

All experiments were analyzed individually as alpha (0,1) designs (Patterson and Williams, 1976) and lattice-adjusted means were used in all subsequent analyses. The program MAPMAKER/QTL (Lincoln *et al.*, 1993) was used to identify putative QTLs

associated with lime-induced chlorosis based on both visual and SPAD data from RILs in EXP1, EXP2, and EXP3 independently. Data from EXP4 were not sufficiently variable to provide significant differences, and will therefore not be presented. The data are presented with LOD scores that represent the log of the odds ratio between the probability that the data would arise from a QTL with this effect divided by the probability that it would arise due to chance alone (Paterson *et al.*, 1988). Visual and SPAD data sets were also analyzed independently for EXP1, EXP2 and EXP3 using the GLM procedure of SAS (SAS, 1989) to determine the association between RFLP loci and lime-induced chlorosis symptoms. Data from all experiments were also subjected to the RIL normal probability analysis of (Brewbaker, 1994), in which an expected distribution for RILs is based on the calculated normal probability curves of the parental inbred sub-lines.

2.4 Results

2.4.1 Phenotypic Data

Chlorosis symptoms for EXP1 appeared within three weeks after planting in most inbred lines, including the Hi31 temperate dent parent of Set G, which closely resembled those reported for iron-deficiency (Choi, 1996). There were no symptoms of chlorosis in the other parent, the Ki14 flint inbred line from Thailand. Symptoms of the 100 RILs varied widely and beyond the parental extremes, some RILs showing mild interveinal chlorosis and other RILs being fully chlorotic and stunted in growth. The first and second visual ratings were significantly correlated (R^2 = 0.91), and averages are presented in Table 2.2.

Table 2.2 Means and standard deviations of SPAD readings and visual ratings for symptoms of chlorosis in parental lines and RILs of Set G.

	EXP	Hi31	Ki14	Means	Range
SPAD	1	12.0±2.8	32.0±2.0	19.0±7.6	4.6 - 34.0
	2	8.0±1.2	31.0±3.9	12.0±7.2	3.0 - 33.0
	3	6.0±1.7	16.0±1.9	9.0±4.1	3.3 - 19.0
	4	52.0±2.8	52.0±1.5	49.0±4.8	33.0 - 57.0
VISUAL *	1	7.6±0.73	2.2±0.65	6.3±2.0	1.7 – 9.0
	2	4.4±0.21	2.1±0.38	4.1±0.80	1.8 – 5.0
	3	3.5±0.27	2.3±0.37	3.5±0.77	1.7 – 4.9
	4	1.5±0.38	1.1±0.20	1.6±0.73	1.0 – 3.5

**Visual ratings on scale of 1-9,(1= green, 9=totally chlorotic) for EXP1 and 1-5 (1= green, 5=totally chlorotic) rating scale for EXP2, EXP3 and EXP4*

The susceptible parent Hi31 had an average visual rating of 7.7 ± 0.73 and the tolerant parent Ki14 had an average of 2.2 ± 0.7. Average visual rating of the 100 RILS varied from 1.5 to 9.0, and the overall average rating was 6.3 ± 2.0. Average SPAD readings for the susceptible and tolerant parents Hi31 and Ki14 were 12.0 ± 2.8 and 32.0 ± 2.0, respectively (Table 2.2). Average SPAD reading of the 100 RILS varied from 4.6 to 34.0, and the overall average reading was 19.0 ± 7.8. SPAD readings were significantly correlated with visual scores ($R^2 = 0.93$).

Among the nine parents of Hawaiian RILs included as checks in this trial, one (Tzi17) was susceptible to soil iron deficiency while the others were intermediate to Hi31 and Ki14 for both visual ratings and SPAD readings.

In the second series of experiments (EXP2, EXP3 and EXP4), 91 Set G RILs out of the original population of 100 were grown at three levels of Fe stress (Table 2.2). EXP2 was grown at moderate stress similar to that of EXP1 (Table 2.1), and data from these experiments showed the highest correlation ($R^2 = 0.83^{**}$) among all experiments between SPAD and visual data. EXP3 was considered a high stress soil with extremely low Fe content (Table 2.1). Visual and SPAD data from this soil (Table 2.2) correlated highly with those of EXP1 ($R^2 = 0.62^{**}$ and $R^2 = 0.83^{**}$) and with that of EXP2 ($R^2 = 0.73^{**}$ and $R^2 = 0.77^{**}$). Under the high stress conditions of EXP3, all inbred lines were chlorotic and stunted and none looked as healthy as CIMMYT open-pollinated populations that had been bred under similar conditions. The difference in EXP3 between parental inbreds Hi31 and Ki14 was significant, averaging 6.3 ± 1.7 and 16 ± 1.9 SPAD units respectively. The RILs in EXP3 averaged 9.1 ± 4.1 SPAD units and ranged from

3.3 to 18 units. EXP4 was grown as a check under low iron stress and seedlings showed no symptoms of chlorosis.

2.4.2. QTL Analysis of Lime-Induced Chlorosis

QTL analysis was performed for visual and SPAD readings using MAPMAKER/QTL and a LOD score threshold of 3.0 for each experiment (Table 2.3). Intervals significantly associated with chlorosis occurred on chromosomes 2, 3, 4, 5, 7 and 9. The interval between RFLP markers *csu*30 and *bnl*5.37 on the long arm of Chromosome 3 gave the highest LOD score values in EXP2 and EXP3 for both SPAD and visual ratings, while the neighboring interval between *csu*30 and *umc*26 on Chromosome 3 (bin 3.05) had the highest LOD score in EXP1 for both rating systems. A region on chromosome 4 between *umc*156 and *umc*200 also appeared to be strongly associated with the symptoms of chlorosis.

Single factor analysis of variance performed with the GLM procedure of SAS revealed 21 RFLP markers accounting for 10% or more of the variation of symptoms of chlorosis, as revealed by SPAD readings and visual data (Table 2.4). Nine of these markers were on Chromosome 3, further confirming the significance of this linkage block. Marker *umc*26 explained 41% of the variation in SPAD readings in EXP1, EXP2 and EXP3 and 51% of the variation in visual data from EXP1. Notably, it also accounted for a high proportion of the variation in EXP3 (R^2 = 38%) under the most severe degree of chlorosis, where many other markers declined in importance. EXP1 also highlighted the significance of marker *umc*26 on chromosome 3. Visual data from EXP2 and EXP3

Table 2.3. Intervals significantly associated with SPAD and visual scores of lime-induced chlorosis. A LOD score of 3.0 was considered the threshold for significance.

		LOD SCORES					
		SPAD			VISUAL		
Interval	**Chrom.**	**EXP1**	**EXP2**	**EXP3**	**EXP1**	**EXP2**	**EXP3**
*umc*122–*umc*131	2	5.87	8.06	5.44	5.32	8.15	6.50
*umc*121-*umc*102	3	ns*	6.45	4.24	ns	6.33	5.40
*csu*30–*umc*26	3	10.72	ns	ns	10.37	ns	ns
*csu*30-*bnl5.37*	3	ns	13.91	8.12	ns	13.04	7.81
*umc*156–*umc*200	4	ns	12.58	9.51	7.06	10.32	6.07
*bnl*5.17- *umc*43	5	9.88	6.24	ns	4.19	4.62	ns
*umc*43–*umc*54	5	ns	ns	4.64	ns	ns	ns
*umc*110–*bnl*4.07	7	ns	5.19	4.68	ns	4.55	ns
bnl 4.07–*umc*35	7	5.33	ns	ns	5.76	7.49	5.51
*umc*190–*npi*209	9	6.33	7.97	5.61	6.27	ns	7.16

* ***ns***= *no significant value generated for that interval*

Table 2.4. R^2 values between marker loci and SPAD readings or visual symptoms of chlorosis as identified from single factor analysis of variance.

		R^2 (%)				
		SPAD			VISUAL	
LOCUS	bin*	EXP1	EXP2	EXP3	EXP1	MEAN
*umc*131	2.05	22.5	28.2	19.5	27.8	24.5
*umc*122	2.07	24.0	32.3	21.7	22.4	25.1
*csu*16	3.02	26.3	28.4	21.6	24.7	25.2
*php*20042	3.02	27.7	23.8	15.7	21.5	22.1
*umc*50	3.04	29.3	34.4	14.5	20.0	24.5
*csu*30	3.05	28.7	24.2	ns**	24.9	25.9
*umc*26	3.05	37.2	46.9	38.1	51.1	43.3
*php*20508	3.05	36.0	26.2	ns	31.6	31.2
*bnl*5.37	3.06	27.1	25.5	12.8	23.2	22.1
*bnl*1.297	3.07	19.8	22.7	21.6	20.8	21.2
*umc*63	3.09	18.6	22.0	24.1	22.0	21.6
*php*20725	4.02	19.8	21.0	17.4	21.0	19.8
*umc*200	4.03	16.8	26.4	17.2	17.8	19.5
*umc*156	4.06	15.7	24.7	16.3	17.2	18.4
*umc*19	4.06	22.1	21.9	21.1	20.4	21.3
*csu*173	5.05	16.0	28.7	ns	14.3	19.6
*bnl*8.39	7.04	25.0	25.8	26.4	27.3	26.1
*php*20075	9.03	19.3	23.7	13.6	14.2	17.7
*umc*190	9.04	23.2	27.0	11.1	20.7	20.5
*bnl*5.09	9.06	24.1	29.9	20.9	25.0	24.9
*csu*50	9.08	22.4	31.5	28.4	31.7	28.5

**bin is an interval between two fixed Core Marker loci*

****__ns__*= no significant value (p=.005) generated for that locus in the analysis*

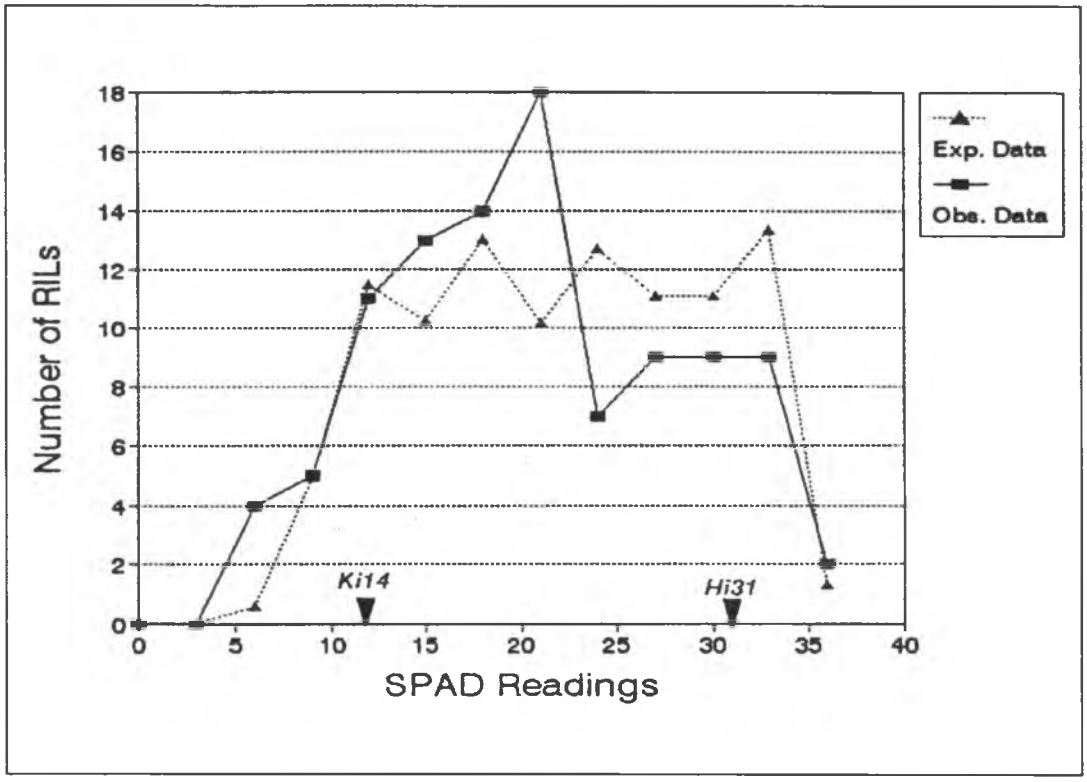
were not significantly associated (R^2 >10%) with markers, and these data are not included here.

Data subjected to the RIL normal probability analysis of Brewbaker (1994), did not fit a single gene model. However, the observed data were approximated by a two-gene model (Figure 2.1). SPAD data in the figure are those of EXP1, for which the Chi-square for goodness of fit was not significant (P = 0.10 - 0.25). The Chi-squares for goodness of fit for EXP2 and EXP3 gave P values of 0.01 - 0.05* and 0.25 - 0.50 ns, respectively. The model assumes that there are two unlinked QTLs of major effect that are segregating in the RILs such that each of the four classes (two parental and two recombinant) are equally represented and the two recombinant classes are intermediate to the parents on a simple additive scale.

2.5 Discussion

A major QTL associated with lime-induced chlorosis in maize was identified in this study on the long arm of Chromosome 3. This corroborates the translocation study of Champoux et al. (1988) that identified the long arms of Chromosomes 3 and 8 as sites of major genes for tolerance. The present study further showed that the RIL segregations of SPAD readings for chlorosis were closely approximated by a statistical model invoking two major QTLs for tolerance (Figure 2.1). It is evident that the major QTL on chromosome 3 associated with lime-induced chlorosis might be at or near the chromosome 3 location of the yellow stripe mutant of maize, *ys*3. This mutant is thought to affect the plant's capacity to secrete phytosiderophores, leading to interveinal chlorosis under Fe-limiting conditions (Basso *et al.*, 1994). Our study did not identify any QTL on

Figure 2.1 Distributions of SPAD for EXP1 with segregation expected on the basis of two major QTLs.



Chromosome 8, but putative minor QTLs occurred on Chromosomes 2, 4, 5, 7, and 9. The study by Champoux et al. (1988) used visual ratings of chlorosis and stunting of plant height as indices, and identified also regions on Chromosomes 2, 4, 6 and 10 as affecting tolerance (Champoux *et al.*, 1988).

Tolerance to lime-induced chlorosis probably involves the plant's ability to respond to multiple factors that interact to provide tolerance. These would include responses to pH and to soil levels of Fe, Ca, and Zn. The complexity of this trait and subsequent plant responses would help to explain why regions on several chromosomes have been associated with lime-induced chlorosis in each of these studies. Complete agreement between such studies is unlikely since genetic material, environments of study, methods of assessing damage, and mapping methodology all differed.

The fields at the Tlaltizapán station that were used during the 1997 wet season had similar pH values (average 7.4) and very high in levels of Ca (above 6500 ppm). This included field EXP4 with highest iron availability, at which little chlorosis was observed. Soil nitrogen and organic matter concentrations were similar for EXP3 and EXP2 (low and medium iron availability, respectively), but approximately 10% higher for EXP4 (high iron availability). This small difference in soil nitrogen availability may have had a limited effect on leaf yellowing. However, severe nitrogen deficiency usually causes uniform leaf yellowing followed by necrosis of lower leaf blades, quite distinct from the interveinal chlorosis that is caused by severe iron deficiency. The major differentiating factor between fields in the 1997 wet season thus was concluded to be iron concentration.

Plant strategies for overcoming iron stress are evidently complex. Phytosiderophores form in maize roots and act to chelate the ferric (Fe^{3+}) ion and make it

possible for the plant to use this form of iron (Choi, 1996). Although iron is not part of the chlorophyll molecule, its catalytic action is vital to the production of this pigment (Olson and Lucas, 1967). Liquid nutrient evaluation under controlled levels of iron as described by Choi (1996) might help determine the physiological roles of QTLs identified in these experiments and specifically their direct relationship to iron stress. Elucidating the most important QTLs should maximize the efficiency of any marker-assisted selection programs for iron-deficiency tolerance. A QTL closely linked to the expression of the trait will allow for selection of superior germplasm prior to expensive and time-consuming field screening.

The Set G has been expanded to about 250 RILs that are planned for future genotyping and field evaluations. This increased population should allow precise identification of major QTLs affecting lime-induced chlorosis and other traits in this germplasm.

CHAPTER THREE

GENETIC DIVERSITY AND ITS RELATIONSHIP TO HYBRID PERFORMANCE IN TROPICAL SUPERSWEET CORN AS REVEALED BY SSR MOLECULAR MARKERS.

3.1 Abstract

Ten supersweet maize inbreds of diverse origin were characterized with 49 SSR molecular markers. Of theses, nine were of tropical origin from Hawaii, Thailand and Australia and one was from Iowa. All of the SSR markers were polymorphic, producing an average of 4 variants per marker. Based on the diversity of the molecular marker analysis, genetic similarity values were calculated for the inbreds. Cluster analysis with the UPGMA algorithm of NTSYS was used to produce a dendrogram, which elucidated four sub-groups among the 10 inbreds. The most distantly related inbred to all others was a Thai inbred that was 53% similar on average. The most similar inbreds, 92%, were two inbreds from Hawaii derived from the same sister line 4 generations ago. There was a strong relationship between molecular based genetic similarity values and the known pedigree information .

The nine tropical lines were intercrossed to create a partial diallel population without reciprocals. This diallel population was evaluated in one location in Thailand and two locations in Hawaii during the spring and summer of 1999 for plant height, ear height, days to silking and anthesis, row number, ear length. Ratings for tenderness and sweetness were completed in the Hawaii trials only. Location effects were highly significant for plant height, ear height, and flowering ($F>>.01$). Heritabilities were high for tenderness (74%), Row number (55%), and sweetness (47%) and all GCA effects were significant ($F > .01$). Heritabilities of plant and ear height, and days to

silking and anthesis were somewhat lower than reported from previous studies (Hallauer and Miranda , 1981), which was attributed to the significant location effects and interactions (F >> .01). Tenderness ratings and row numbers were not significantly affected by location.

Correlations (r) between GS values with SCA estimates were highest for days to silking and anthesis, plant and ear height, and ear length at (72%, 69%, 68%, 51%, and 49%, respectively) and were all highly significant (p>.01). Tenderness ratings and GS values were also significantly correlated (p = 0.05). Sweetness and row number were not significantly correlated with GS values based on SSR analysis in this study. The GS values provided useful information about the diversity among the inbreds and insight into potential for hybrid performance for most of the traits evaluated in this study. The study also demonstrated the importance of in-field bite-test evaluations of tenderness and sweetness, and showed that these evaluations are enhanced by sib-pollinations of the lines under evaluation.

3.2 Introduction

Maize, *Zea m*ays L., is among the most diversely utilized cultivated crops due to the genetic diversity within the species. Sweet corn is a vegetable crop of maize, although young corn or 'green corn' of many endosperm genotypes has been consumed in all corn growing countries. The origin of sweet corn dates back to the pre-Columbian times and utilization of the endosperm mutant sugary, *su*, was the first endosperm mutation incorporated to produce a vegetable corn (Boyer and Shannon, 1983). Most of the other endosperm mutants were discovered in the early half of the 20th century. Sweet corn consumption the 1900s has been primarily in North America, but the popularity is

growing worldwide (Tracy, 1994). This expansion of sweet corn production has led to a increased need for knowledge of not only specific endosperm mutants and other genes, but also of breeding strategies and technologies to aid in the effort of producing superior sweet corn germplasm for growers and consumers.

The sweet characteristic is based primarily on recessive endosperm mutants that accumulate sugars in the corn kernel (Table 3.1). These mutants are used singly or in combination to alter the carbohydrate levels in the endosperm of maize kernels. The utility and function of these endosperm mutants have been the subject of research in defining the biochemical action of starch mutants (Singletary *et al.*, 1997).

Sweet corn is utilized as both a fresh and processed vegetable. As a consequence the breeding of superior sweet corn has focused both on quality and appearance of the ears as well as more traditional objectives of yield and agronomic performance (Tracy, 1994). The complexity of selection criterior for superior sweet corn limits the germplasm conforming to commercial standards, a factor contributing to the narrow germplasm base associated with most modern sweet corn (Revilla and Tracy, 1997). The characters studied in the development of superior sweet corn germplasm include flavor components such as sweetness, tenderness, aroma, texture, and post-harvest quality. More "traditional" objectives include improved germination and emergence, disease and insect resistance, stress tolerance, improved plant morphology and high yield.

Sweetness has long been considered the most important constituent of flavor as perceived by the consumer (Culpepper and Magoon, 1924). Many evaluations have been published on the component carbohydrates associated with the different kernel mutants

Table 3.1 Endosperm mutants of corn affecting carbohydrate composition of the kernel (Boyer and Shannon, 1983)

Gene	Gene Symbol	Chromosome	Kernel Phenotype
amylose extender	ae	5	Tarnished, translucent, or opaque; sometimes semifull
brittle	bt	5	Shrunken, opaque to tarnished
brittle-2	bt2	4	Shrunken, opaque to tarnished
dull	du	10	Opaque to tarnished; semi-collapsed translucent with some opaque sectors
floury	fl	2	Opaque
shrunken	sh	9	Collapsed, opaque
shrunken-2	sh_2	3	Shrunken, opaque to translucent
sugary	su	4	Wrinkled, glassy
sugary-2	su_2	6	Slightly tarnished to tarnished
sugary enhancer	se	2	Light colored, slow drying kernels observed in homozygous su lines only
waxy	wx	9	Opaque

(Juvik *et al.*, 1993; Rosenbrook and Andrews, 1971; Wong *et al.*, 1994). Measuring sugar levels and perceptions of sweetness are complicated selection criterion. Analysis of sugars with high pressure liquid chromatography (HPLC) (Doehlert *et al.*, 1993; Tadmor *et al.*, 1995) and gas liquid chromatography (GLC) (Carey *et al.*, 1984; Ferguson *et al.*, 1978; Juvik and LaBonte, 1988) are often out of the scope of most breeding programs as a practical selection tool. Analysis of aromas and other sensory characteristics are also technically difficult and time-consuming (Azanza *et al.*, 1996; Hodgins and Simmonds, 1995). Easier, more efficient screening methods that could be applied to large numbers of samples would be of great benefit to sweet corn breeders (Rosenbrook and Andrews, 1971).

Tenderness is a well-established quality factor (Culpepper and Magoon, 1924) and is most commonly associated with pericarp thickness (Tracy and Schmidt, 1987). Pericarp thickness has been shown to be a polygenic trait controlled by 1.4 to 5.9 genes (Ito and Brewbaker, 1991). Although pericarp thickness can be a useful index for selection of tenderness it is noted that other criterion like a kernel bite test (Ito and Brewbaker, 1981) further enhance the selection for improved tenderness. Thin pericarps can crack and can be damaged in shelling. This damage is associated with poor germination and seedling emergence possibly due to electrolyte leakage that leads to a loss of energy for embryo growth and allows pathogen invasion (Zan and Brewbaker, 1999; Wann, 1986).

Germination and seed quality are very important characters in supersweet mutants. The dramatically reduced starch levels coupled with greatly increased sugar concentration leads to an ideal environment for the growth and development of soil borne

pathogens. This can be a problem at the time of germination and throughout the growing season e.g. ear rots. Investigations into seed treatments (Hartz and Caprile, 1995) and germination of the different endosperm mutants (Zan and Brewbaker, 1999) has been useful. Selection for improved seedling emergence based on measurements of field emergence and kernel weight as well as a number of other kernel and plant characteristics is effective (Ito and Brewbaker, 1981; Juvik *et al.*, 1993).

Agronomic characteristics are equally as important in sweet corn germplasm evaluation and improvement. Traits such as kernel weight, kernel depth, kernel row number, ear weight, ear length, plant height, ear height are often measured and evaluated singly or as components of yield (Wong *et al.*, 1994). Disease, insect and other stress tolerances and resistances are always a subject of selection, although the relative importance varies with environments.

The complexity associated with selection for superior sweet corn germplasm is a consequence of many factors; the unique genetic base of mostly recessive endosperm mutations, the diversity of uses, the complexity of quality based on human perceptions, and variation in environments and subsequent biotic and abiotic stresses unique to those environments. The ultimate success of the sweet corn breeding program relies on the ability to maximize production of a quality product under local environmental stresses by incorporating the most suitable germplasm.

Molecular markers like SSRs offer a relatively new method of characterizing germplasm with improved resolution and allowing better definition of the germplasm resources available (Senior *et al.*, 1998). The premise is that diversity on the molecular level should provide a means of fingerprinting germplasm, determining relatedness of

germplasm (genetic distance), help classify germplasm into heterotic groups, and ultimately help to predict hybrid performance (Bernardo, 1992; Godshalk *et al.*, 1990; Livini *et al.*, 1992; Melchinger *et al.*, 1990b). Studies of this type utilizing molecular marker-based estimates of genetic distance as a predictor of hybrid performance have given mixed results, some positive (Lee *et al.*,. 1989, Melchinger 1992) and others with low correlations (Godshalk *et al.*, 1990; Melchinger *et al.*, 1990b). Marsan *et al.*, (1998) found that predictive value was enhanced by studying a diallel population (parents genotyped with RFLPs and AFLPs)

The objectives of this study were (1) to characterize a series of 10 elite supersweet corn inbreds from Hawaii, Thailand, Australia, and Iowa with SSR molecular markers; (2) create a partial diallel series of crosses with the inbreds; and (3) assess the relationship between genetic distance and hybrid performance among these elite supersweet corn inbreds based on the genotypic analysis and diallel cross evaluation.

3.3 Materials and Methods

3.3.1 Germplasm and replicated trials

Ten supersweet inbred lines (Table 3.2) were selected as parents based on previous evaluations of the University of Hawaii. These inbreds included *brittle-1* stocks developed in Hawaii (190, 217, Hi36, Hi37, Hi38), Thailand (bA11, bF47), Australia (Kbtl13, Kbtl32) and one *shrunken-2* inbred from Iowa ($Ia453sh_2$). The inbreds represented an array of traits associated with quality and plant morphology. Of the inbreds from Hawaii developed by Brewbaker, 217 and 190 were derived from a selection effort for enhanced tenderness and sweetness and are from the same sister lines four generations ago. The Hi36, Hi37 and Hi38 inbreds were all selected out of HS#9, an

Table 3.2. Description of parental inbred lines.

Inbred	Origin	Seed Source	Special Characteristics (in Waimanalo)
217	Hawaii	98-1532	sweet, tender, erect leaves
Hi36	Hawaii	98-229	occasional pericarp deformation
Hi37	Hawaii	98-120	poor roots, tall, tender
Hi38	Hawaii	96-1908	sweet, erect leaves, moderate tenderness
bA11	Thailand	98-1721	unique crispy tenderness, taller & thinner plant
bF47	Thailand	98-1722	very tough, not sweet, tassel tipped ear, nice plant
Kbtl13	Australia	97-497	tender, thin spindly stalk, early maturing
Kbtl32	Australia	97-492	tender, not sweet, erect leaves
190	Hawaii	98-1586	tall, sweet, tender
Ia453sh_2	Iowa	98-149	short, multiple disease susceptibility, weak

open pollinated variety also developed by Brewbaker. The Thai inbreds also come out of HS#9 crossed with composite populations out of Suwan-1 (Thailand) for bA11, while bf47 was selected out of HS#9 crossed with Nicaraguan material. The Kbtl inbreds are based on Hawaiian inbreds, but exact pedigree information is not available.

A partial diallel series of crosses were created in November 1998 using nine of these inbreds. No crosses were possible with the Ia453sh_2 inbred as the plants were very weak and no seed from any of the attempted crosses developed. The resulting diallel population consisted of 36 hybrids and nine inbred parents. The diallel was planted in replicated randomized complete block designs in February 1999 in Thailand (THAI) and June 1999 at the Waimanalo Research Station on Oahu (WAI-1, WAI-2) and the Mealani Research Station on Hawaii (MEAL). The Thailand trial, THAI, was planted on February 2, 1999 at the Sweet Seeds Co., Ltd., Field Station in Saraburi, Thailand located at approximately 40 MASL and 14° N. Two replications of the nine parental inbreds and 35 of the diallel crosses were planted. There was not enough seed of the bF47 x 190 hybrid and it was not included it in the planting. Plots were 5m long and were sprinkler irrigated as needed, at approximately 5-day intervals. Days to silking (DTS) and anthesis (DTA), plant height (PH) and ear height (EH), kernel row number (RN), ear length (EL), ear weights at harvest, but only comments on tenderness, sweetness and quality aspects of the ears were taken.

The Waimanalo trial (WAI-1), was planted on June 8, 1999 at the University of Hawaii Research Station in Waimanalo, Oahu at 20 MASL and 21°N. Two replications of the nine inbreds and 36 diallel crosses were planted in 5m plots with drip irrigation. This was the only complete trial planted due to short seed supplies of some hybrids. Parental

inbred lines were used in evaluations, but also for seed increase of the diallel crosses. Three sib-pollinations per plot were made to assess ear tenderness and sweetness 18-21 days after pollination. Sweetness and tenderness were evaluated with in-field bite tests using a 1-5 scale, (1 = excellent, 5 = poor). Data were also taken on DTA, DTA, PH (cm), EH (cm), RN , EL (cm), kernel depth , and ear diameter.

A second replicated trial was planted at Waimanalo (WAI-2), on June 30, 1999. Thirteen of the diallel hybrids and the nine parental inbred lines were planted and evaluated in the same way as WAI-1. The trial was incomplete due to limited seed supply.

On June 28, 1998 a fourth trial was planted at the Mealani Research station on Hawaii (MEAL) at 20° N and 940 MASL. There was only sufficient seed for two replications of 26 F1s, but 35 F1s (no bF47 x 190), were included in at least one replication along with the nine parental inbred lines. The same data were collected for this trial as WAI-1 and WAI-2, but the field bite-test evaluations of tenderness and sweetness were done on open-pollinated ears within each plot.

3.3.2 Molecular Marker Analysis.

The molecular marker analysis was conducted at the Plant DNA Fingerprinting Unit located at the Central Laboratory and Greenhouse Complex at the Kamphaengsaen Campus of Kasetsart University in the Nakhon Pathom province of Thailand. DNA was extracted via a CTAB mini-prep extraction from one week old seedlings planted in a greenhouse in January 1999. The 10 parental inbred lines were characterized with 49 SSR

markers covering all 10 chromosomes, selected from the Plant DNA Fingerprinting Unit's collection based on supply of primers.

A standard PCR reaction cocktail of 5 µl was made with the components of 5 ng of template DNA, 1x buffer, 0.4 µl of 25µl MgCl2, 0.2 mM DNTPs, 1µm of each of the forward and reverse primers, 0.19 µl of 1unit/µl TAQ, and 1.91 µl of DH_20. All PCR reactions were run in a 384-well GeneAmp PCR system 9700 thermal cycler (PE Applied Biosystems) using of a single denaturation cycle at 94°C 1 minute, 40 cycles 94°C for 30 seconds, 58°C 30 seconds and 72°C 60 seconds, followed by a cycle at 72° 7 minutes and a final holding temperature of 4° C . PCR products were run on 4% polyacrylimide gels and silver stained. The stained gel plates were scanned into the computer as a permanent record of the results. The bands were scored with a binary approach based on presence and absence of bands at specific locations on the gel.

Genetic similarity (GS) was estimated following Senior *et al.* (1998), using the binary data. This procedure incorporates a simple matching coefficient such that

$$GS = m / (m + n)$$

where m = the number of matches and n = the number of mismatches (Sneath and Sokal, 1973). A result of 0 implies that the lines are completely different and 1 would represent lines genetically similar at all loci.

3.3.3 Statistical Analysis

Analyses of variance of individual trials and of data combined across locations were calculated (Brewbaker, 1994). Diallel analysis was performed on parents and F1 data (without reciprocals), Model I of Method 2 Griffings (1956). General and specific combining abilities (GCA and SCA) were generated (Brewbaker, 1994). Analysis of variance of combining abilities was calculated using the fixed model to estimate GCA and SCA effects, and using the random model to estimate narrow and broad sense heritability estimates.

Associations among the 10 inbreds were determined from cluster analysis based on genetic similarity estimates. The SAHN function of the NTSYS-pc program (Rohlf, 1993) was used to perform the clustering algorithm for unweighted pair group method using arithmetic averages (UPGMA). Matrix comparisons were also calculated producing cophenetic correlations to verify the fit of the clustering method.

3.4 Results

3.4.1 Genetic variation and diversity for SSR Markers

A total number of 176 alleles were detected when evaluating the 10 inbreds with 49 SSR markers. Every one of the SSR markers utilized in this study was polymorphic among the 10 inbreds screened. The level of polymorphism and the level of allele variation are comparable to other SSR results (100% polymorphic) (Senior *et al.*, 1998) and with RFLPs (98% polymorphic)(Melchinger *et al.*, 1990b), 96% (Livini *et al.*, 1992); 91 % in (Marsan *et al.*, 1998). The average number of variants per marker loci was 4 with a range of 2 to 6 variants per marker (Table 3.3). Several of the SSRs amplified

Table 3.3 SSR markers used to characterize the parental inbreds and the number of variants produced by each marker

SSR Marker	Chromosome Location	Number of Variants
bngl 149	1.00	3
phi056	1.01	3
phi097	1.01	2
bngl109	1.02	6
bngl439	1.03	3
bngl 421	1.06	5
bngl 615	1.07	4
phi002	1.08	4
phi038	1.08	2
phi039	1.08	4
phi011	1.09	3
bngl125	2.03	4
nc132	2.04	2
nc133	2.04	3
phi127	2.07	3
phi099	3.02	5
phi029	3.04	3
phi053	3.05	6
phi073	3.05	4
phi047	3.09	6
phi021	4.02	3
nc005	4.04	3
phi026	4.04	4
bngl490	4.05	3
phi006	4.10	3

SSR Marker	Chromosome Location	Number of Variants
phi019	4.10	3
nc007	5.00	3
phi024	5.00	5
bngl1143	5.01	4
bngl653	5.04	3
phi087	5.06	4
phi048	5.07	3
bngl389	5.09	6
bngl249	6.01	5
phi126	6.02	5
nc013	6.05	4
phi123	6.07	4
phi034	7.02	3
phi051	7.06	3
phi014	8.04	3
bngl666	8.05	4
phi015	8.08	2
phi028	9.01	4
phi033	9.01	2
MACT2B8	9.01	4
phi017	9.02	3
phi032	9.04	2
phi040	9.05	3
phi035	10.05	3

more than a single band for each inbred. This could be attributed to remaining heterozygosity in the inbreds, contamination, or amplification of similar sequences in two separate regions of the genome. Such duplication has been well documented in the maize genome (Helentjaris, 1995).

The average GS value was 0.61 (Table 3.4). The inbreds 217 and 190 were the most similar with a GS value of .92 and Kbtl13 and bA11 the most divergent with a GS value of 0.48. The similarity of 217 and 190 was supported by pedigree information as they were selected from sister lines 4 generations back. The array means were also presented (Table 3.4) indicating that overall, 190 was the most similar of all other inbreds, array mean GS = 0.67. The inbred bA11 was the most divergent with an array mean GS = 0.53. The second most divergent inbred was Ia453sh_2 was with mean GS = 0.55.

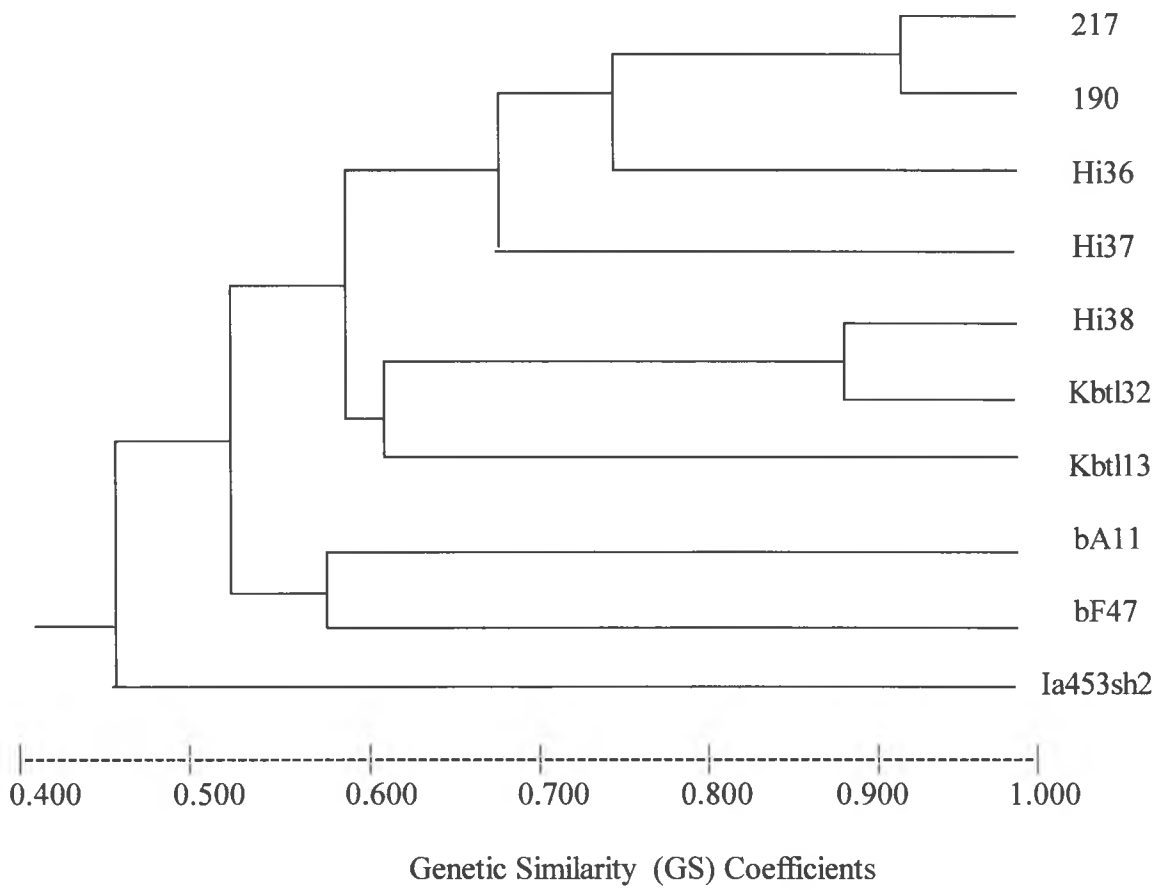
A dendrogram generated from analysis of the GS values used the UPGMA clustering algorithm to graphically display GS relationships of the inbreds (Figure 3.1). The dendrogram had a high cophenetic correlation of r = 0.95. A cophenetic value of r > 0.9 is interpreted to be a very good fit (Rohlf, 1993).

The relationship of the inbreds in Figure 3.1 demonstrated that the single temperate *sh_2* inbred, Ia453sh_2, was more distantly related to all of these lines that originate from the tropics. Lines that make a superior Thai hybrid bA11 and bF47, had 58% similarity. Hawaiian supersweet hybrid, HS#10 [(Hi36 x Hi37) x Hi38] also produced an excellent hybrid with similarities of (.73%) x (~60%). Four groups emerge in Figure 3.1, one with Hawaiian inbreds (217, 190, Hi36, Hi37), another of Hawaiian

Table 3.4 Calculated genetic similarity values for binary data.

	217	Hi36	Hi37	Hi38	bAll	bF47	Kbtl13	Kbtl32	190	Ia453sh$_2$	ARRAY MEANS
217		0.74	0.66	0.62	0.52	0.56	0.63	0.57	**0.92**	0.49	0.63
Hi36			0.73	0.59	0.56	0.57	0.56	0.60	0.78	0.49	0.62
Hi37				0.62	0.53	0.58	0.61	0.62	0.70	0.56	0.62
Hi38					0.50	0.60	0.62	0.89	0.66	0.59	0.63
bA11						0.58	**0.48**	0.54	0.52	0.50	0.53
bF47							0.57	0.61	0.56	0.55	0.59
Kbtl13								0.63	0.66	0.61	0.60
Kbtl32									0.61	0.62	0.64
190										0.50	0.67
Ia453sh$_2$											0.55

Figure 3.1. Association among inbred parents revealed by UPGMA cluster analysis of genetic similarity coefficients from SSR data.



and Australian inbreds (Hi38, Kbtl13, Kbtl32), a third with the Thai inbreds (bA11and bF47) and the fourth with the Iowa inbred ($Ia453sh_2$).

3.4.2 Diallel analysis of phenotypic traits

The most tender parent was 217 followed by 190 and bA11 with average tenderness rating of 1.5, 1.6, and 2.3 respectively (Table 3.5). The toughest parent was bF47 with an average 4.7 tenderness rating. The sweetest parents were 190, 217, and bA11 with ratings of 1.3, 1.6, and 2.1 respectively. The earliest flowering parent was Kbtl13 with DTS and DTA averages of 64 and 63 days . The latest flowering with DTS and DTA of 70 and 69 days were parents 217 and bF47. Parent bF47 was the shortest inbred with an average PH and EH of 143cm and 47cm respectively. Row numbers ranged from 10 to 16 for the parents, bF47 and Kbtl32 averaged 11 while Hi36 had an average of 16 rows. Although we expect only even row number values the odd number average represents the variation within the replications and locations. The shortest average ear length was bA11 with a 12cm average ear length while Kbtl32 had the longest average ear length of 16cm.

Most tender hybrids were 217 x Hi37, 217 x Hi36, 217 x 190 and Hi36 x 190 with a 1.8 average rating, followed closely by Kbtl13 x 190 and 217 x Kbtl13 both the average ratings of 2.0 (Table 3.6). The least tender hybrids were those with bF47 as a parent, with average tenderness ratings ranging from 3.8 (bF47 x Kbtl32) to 4.6 (Hi36 x bF47). Only the bF47 x 190 and bF47 x Kbtl13 hybrids had a lower rating of 3.5 and 2.5 respectively. When comparing the bF47 crosses with the other cross combinations the bF47 hybrids

Table 3.5 Summary of average values of each parent for each trait combined across locations.

Parent	Tender	Sweet	DTS	DTA	PH	EH	RN	EL
217	1.5	1.6	70	69	115	55	13	12
hi36	2.3	3.0	67	65	128	53	16	14
hi37	3.8	2.7	67	65	131	55	15	15
hi38c	3.4	2.8	68	68	122	46	12	15
ba11	2.3	2.1	68	68	138	61	15	12
bf47	4.7	3.3	70	69	106	47	11	13
Kbtl13	3.1	3.1	64	63	143	56	12	14
Kbtl32	3.0	2.6	69	66	119	45	11	16
190	1.6	1.3	68	67	120	54	14	13

Table 3.6 Diallel table for tenderness ratings means.

	217	Hi36	Hi37	Hi38	bAll	bF47	Kbtl13	Kbtl32	190	ARRAY MEANS
217	1.50	1.79	1.63	2.33	1.96	3.75	1.96	2.25	1.79	2.18
Hi36		2.33	3.46	3.88	3.17	4.58	2.96	3.46	1.83	3.14
Hi37			3.79	3.58	3.13	4.54	3.00	3.25	1.96	3.07
Hi38				3.38	3.08	4.13	3.42	2.91	2.42	3.22
bA11					2.29	4.38	2.29	2.88	2.46	2.92
bF47						4.67	2.46	3.75	3.53	3.44
Kbtl13							3.13	2.58	1.96	2.58
Kbtl32								3.04	2.48	2.94
190									1.66	2.30

Table 3.7 Diallel table for sweetness ratings means.

	217	Hi36	Hi37	Hi38	bAll	BF47	Kbtl13	Kbtl32	190	ARRAY MEANS
217	1.55	2.71	2.25	2.54	1.95	2.58	1.83	2.80	1.83	2.31
Hi36		3.00	3.17	3.46	3.46	3.11	2.88	3.21	2.29	3.03
Hi37			2.67	2.92	2.71	2.46	2.54	2.50	2.38	2.61
Hi38				2.75	1.75	2.42	2.71	2.41	1.92	2.51
bA11					2.08	3.23	2.33	2.17	2.21	2.47
bF47						3.25	2.13	2.50	2.45	2.55
Kbtl13							3.13	1.46	1.50	2.17
Kbtl32								2.63	2.38	2.43
190									1.34	2.12

had the highest array mean of 3.44. The Hi38 inbred was the second toughest parent in all crosses with an array mean tenderness rating of 3.22.

The sweetest hybrids were Kbtl13 x Kbtl32 and Kbtl13 x 190 with sweetness ratings of 1.5, and 217 x Kbtl13, 217 x 190, Hi38 x bA11 all had average rating of 1.8(Table 3.7). Inbred 190 made the sweetest hybrids with an array mean sweetness rating of 2.12, followed closely by Kbtl13 and 217 with array mean values of 2.17 and 2.31 respectively (Table 3.6). The least sweet hybrid series was the Hi36 series with an array mean value of 3.03 and a range of 2.71 to 3.46 (Table 3.7). Those hybrids that were the sweetest and most tender in combination were 217 x 190, 217 x Kbtl13, Hi36 x 190, Kbtl13 x 190.

The earliest flowering hybrids were Hi37 x bAll and bAll x Kbtl13 with 60 DTS and Hi37x Kbtl13, bF47 x Kbtl3 and Hi36 x bA11 at 61 DTS (Table 3.8). Inbreds bA11, Kbtl13, and Hi36 produced the earliest hybrids (Table 3.9), while 217 produced the latest flowering hybrid series (Tables 3.7 and 3.8).

The tallest hybrids were Hi36 x bA11 and bA11 x Kbtl13 with 185cm and 183cm respectively. Overall bA11 hybrids were the tallest with an array mean of 172cm. Hi38, Kbtl32 and bF47 hybrids were the shortest with an average plant height among the hybrids of 147cm, 149cm, and 150cm (Table 3.10). The highest EH values were 87cm and 84cm for bA11 x 190 and Hi36 x bA11 respectively. The inbred bA11 produce hybrids with the highest ears (array mean EH = 78cm), and Kbtl32 and Hi38 had the lowest ears with array mean ear heights of 61cm and 62cm (Table 3.11).

The average row numbers ranged from 11 to 18 rows (Table 3.12). The hybrid with the smallest average row number was bA11 x Kbtl32 that had 11 rows and the

Table 3.8 Diallel table for days to silking (DTS) means.

	217	Hi36	Hi37	Hi38	bAll	bF47	Kbtl13	Kbtl32	190	ARRAY MEANS
217	70	65	67	66	63	68	63	66	70	66
Hi36		67	63	62	61	63	62	63	65	63
Hi37			67	65	50	64	61	65	65	63
Hi38				68	62	64	63	69	66	65
bA11					68	65	60	62	62	62
bF47						70	61	65	65	64
Kbtl13							64	64	63	62
Kbtl32								69	64	65
190									68	65

Table 3.9 Diallel table for days to anthesis (DTA) means.

	217	Hi36	Hi37	Hi38	bAll	bF47	Kbtl13	Kbtl32	190	ARRAY MEANS
217	69	64	66	64	64	66	63	64	68	65
Hi36		65	62	61	62	63	62	62	64	62
Hi37			65	63	61	63	61	63	64	63
Hi38				68	62	64	62	67	64	63
BA11					68	66	61	62	63	63
bF47						69	61	63	63	63
Kbtl13							63	62	62	62
Kbtl32								66	63	63
190									67	64

Table 3.10 Diallel table for plant heights (PH) means.

	217	Hi36	Hi37	Hi38	bAll	bF47	Kbtl13	Kbtl32	190	ARRAY MEANS
217	115	152	148	154	165	147	156	153	129	151
Hi36		128	150	154	185	152	161	152	154	156
Hi37			131	152	174	142	153	151	152	153
Hi38				122	161	140	145	117	151	147
BA11					138	160	183	172	174	172
bF47						106	162	144	149	150
Kbtl13							143	149	167	159
Kbtl32								119	154	149
190									120	154

Table 3.11 Diallel Table for ear height (EH) means.

	217	Hi36	Hi37	Hi38	bAll	bF47	Kbtl13	Kbtl32	190	ARRAY MEANS
217	55	70	64	61	76	66	66	63	59	66
Hi36		53	68	68	84	74	63	62	78	71
Hi37			55	64	80	64	62	60	74	67
Hi38				46	71	59	61	45	65	62
BA11					61	73	81	76	87	78
bF47						47	74	57	68	67
Kbtl13							56	60	74	68
Kbtl32								45	64	61
190									54	71

Table 3.12 Diallel table for row number (RN) means.

	217	Hi36	Hi37	Hi38	bAll	bF47	Kbtl13	Kbtl32	190	ARRAY MEANS
217	13	17	14	13	15	14	13	13	14	14
Hi36		16	17	15	18	16	16	14	17	16
Hi37			15	13	16	14	13	14	16	15
Hi38				12	14	12	13	13	14	13
BA11					15	15	14	11	15	15
BF47						11	15	13	13	14
Kbtl13							12	12	13	14
Kbtl32								11	13	13
190									14	14

Table 3.13 Diallel Table for ear length (EL) means.

	217	Hi36	Hi37	Hi38	bAll	bF47	Kbtl13	Kbtl32	190	ARRAY MEANS
217	12	19	18	19	17	18	18	19	15	18
Hi36		14	18	19	17	20	19	20	19	19
Hi37			15	18	18	16	20	20	19	18
Hi38				15	18	18	19	18	18	18
bA11					12	16	18	19	17	18
bF47						13	19	19	18	18
Kbtl13							14	18	19	19
Kbtl32								16	19	19
190									13	18

greatest row number was 18 for hybrid Hi36 x bA11. Across all hybrids inbred Kbtl13 produced the lowest row number hybrids (array mean RN=13 rows) and Hi36 produced the hybrids with the highest row number with an array mean of 16 rows (Table 3.12).

Ear lengths ranged from 15cm to 20cm (Table 3.13). 217 x 190 was the shortest hybrid and the longest were Hi36 x bF47, Hi37 x Kbtl32, and Hi37 x 190 all with 20cm average ear length . On average Kbtl32 and Hi36 produce the longest ears while bA11 produced the hybrids with the shortest average ear lengths (Table 3.13)

ANOV of the parents, hybrids and combined analysis for each trait are presented in Tables 3.14-3.16. Values significant at the 0.05 level (in bold) and those significant at the 0.01 level (bold and italic). The degrees of freedom for the tenderness and sweetness analysis were different as these were only evaluated at two locations and all other traits were measured at three locations.

All parents were significantly different (Table 3.14) and there was a large location effect associated with DTS, DTA, PH, and EH on the parental inbreds (F >.01). There was no significant effect of location on the tenderness and sweetness ratings for the parents (Table 3.14). There was also a significant location effect (F >.01) associated with DTS, DTA, PH and EH in the hybrid ANOVs. Tenderness and sweetness ratings were not significantly effected by location among the hybrids (Table 3.15).

From the combined hybrid ANOV the location effect was significant for all traits except tenderness and row number (Table 3.15) and suggested a significant environmental effect on DTA, DTA, PH, EH, EL and sweetness. The location effect was largely due to the Mealani trial. The higher elevation of 940m (compared to 20m and 40m for Waimanalo and Thailand) and cooler temperatures at Mealani led to a more than

Table 3.14 Summary of mean square values for all traits based on fixed model from combined parental ANOV.

	df†	Tender	Sweet	df††	DTS	DTA	PH	EH	RN	EL
TOTAL	35	1.20	0.65	53	323	314	774	431	4.15	0.46
Locations	1	0.00	0.01	2	***8312****	***8017***	***15341***	***9315***	0.35	0.40
Reps in Loc.	3	0.52	0.05	3	5	14	199	148	0.65	0.17
Parents	8	***3.44***	***1.56***	8	**16****	**23**	***790***	***164***	***22.96***	***1.28***
Par x Loc.	8	0.76	0.49	16	13	12	***147***	**97**	***1.55***	***0.70***
ERROR	15	0.44	0.39	24	6	7	46	37	0.37	0.06

† *Tenderness and sweetness analysis only for WAI and MEAL locations.*

†† *All other traits combined across THAI, WAI and MEAL trials.*

* *Bold italics indicate significance at .01*

***Bold indicates significance at .05*

Table 3. 15 Summary of mean square values for all traits based on the fixed model from combined hybrid ANOV.

	df†	Tender	Sweet	df††	DTS	DTA	PH	EH	RN	EL
TOTAL	143	1.02	0.56	215	315	304	695	494	3.13	0.91
Locations	1	0.28	2.26	2	***32801****	***32049***	***39301***	***38553***	0.17	2.15
Reps in loc.	2	1.67	3.32	5	28	16	1524	486	0.85	0.82
Hybrids x loc	35	0.38	0.46	70	***10***	*7*	206	93	1.26	1.39
HYBRIDS	35	***2.79***	***1.08***	35	***32***	***16***	***1039***	***432***	***13***	***2.14***
ERROR	35	0.50	0.28	103	3	2	121	50	1.16	0.16

† *Tenderness and sweetness analysis only for WAI and MEAL locations.*
†† *All other traits combined across THAI, WAI and MEAL trials.*
* *Bold italics indicate significance at .01*

30 day delay in both DTS and DTA and a decrease in PH and EH of as much as 30% and 50% respectively. The decreases in plant height and sweetness can be associated with the lower temperatures. Tenderness had a significant effect from the interactions of entries, hybrids and parents by location where sweetness had a significant location effect (F >.01), but interactions were not significant. Row number was the only trait not affected significantly by locations or interactions with location. For all traits entries, hybrids, parents and hybrids vs parents were significantly different from each other (Table 3.16). There was clear heterosis exemplified by an increase of the hybrid averages compared to the parental averages for all traits (Table 3.17). The effects on sweetness and tenderness were less evident with only a 0.04 and 0.03 improvement in the hybrids compared to the inbreds. Among the hybrids there was a decrease in DTS and DTA of four and three days respectively among the hybrids. There was an increase in average PH and EH of 30cm and 15cm compared to parental averages. Ear lengths were increased by almost 5cm above the parental average in the hybrid progeny (Table 3.17).

The highest CVs were associated with the sweetness and tenderness data, 23% and 19% respectively. However, tenderness CVs were considerably lower than the findings of Ito (1981) who reported CVs of 26.1%-32.4% for bite-test tenderness ratings. Ito suggested than an increase in the rating scale to 1-7 and the use of controlled pollinations would diminish the variability. In the Waimanalo (WAI) trial there were controlled pollinations and the overall CV was 22% and 14% for sweetness and tenderness respectively, compared to the open pollinated evaluations at Mealani (MEAL) which had 27% and 20% CV's, respectively (Table 3.18). There were no reported CV's for sweetness bite-test evaluations for comparison.

Table 3.16 Summary of mean square values for all traits based on the fixed model for combined ANOV.

	df†	Tender	Sweet	df††	DTS	DTA	PH	EH	RN	EL
Total	179	1.09	0.59	269	318	542	853	518	3	0.94
Entries	44	***2.99****	*1.15*	44	***45***	***39***	***1854***	***634***	***15***	***4***
Hybrids	35	***2.79***	***1.02***	35	***32***	***32***	***1039***	***458***	***13***	***2***
Parents	8	***3.44***	***1.56***	8	***16***	***10***	***790***	***164***	***22***	***1.28***
H vs P	1	***6.10***	***2.45***	1	***723***	***295***	***38876***	***10173***	***38***	***98***
Locations	1	0.60	***1.80***	2	***10291***	***5730***	***14233***	***12105***	0.79	***0.62***
E x L	44	***0.78***	0.45	88	***711***	***1572***	***1109***	***948***	1.28	***0.53***
H x L	35	***0.76***	0.43	70	*10*	**23**	206	99	1.26	***1.39***
P x L	8	***0.76***	0.49	16	*13*	*26*	147	97	1.55	**0.70**
(HvsP)xL	1	**1.35****	0.92	2	***30822***	***33489***	***40409***	***35763***	-0.26	-30
Error	90	0.32	0.37	135	4	12	161	63	0.99	0.16

† *Tenderness and sweetness analysis only for WAI and MEAL locations.*
†† *All other traits combined across THAI, WAI and MEAL trials.*
* *Bold italics indicate significance at .01*
***Bold indicates significance at .05*

Table 3.17 Summary data for all locations (THAI, WAI and MEAL) combined for each trait in the study.

TRAIT	Parent AVG	Hybrid AVG	Overall AVG	CV %	h%
Sweet	2.52	2.48	2.48	23	47
Tender	2.95	2.92	2.91	19	74
DTS	68	64	65	3	21
DTA	67	63	64	3	24
PH	125	155	149	9	19
EH	52	68	65	12	22
RN	13	14	14	7	55
EL	14	18	17	6	0.00

Table 3.18 Summary of parent and hybrid averages and the CV of the sweetness and tenderness data in both testing locations, Waimanalo and Mealani.

Values	Sweet		Tender	
	WAI-1	MEAL	WAI-1	MEAL
Parental Average	2.50	2.64	2.87	3.08
Hybrid Average	2.39	2.54	2.85	2.94
CV%	22	27	14	20

The heritabilities of tenderness and sweetness were relatively high in combined analysis, 74% and 47%, respectively (Table 3.18). The heritabilities for DTS, DTA, PH and EH were significantly less than those previously reported by Hallauer and Miranda (1981), who surveyed a number of studies and concluded that heritabilities for these traits were consistently greater than 50% and less than 70%. That range was far greater than the 21%, 24%, 19%, and 22%, found in this study for DTS, DTA, PH, and EH, respectively. This could be attributed to the environmental effect associated with the different locations in this study. The heritability of row number in this study of 55% in combined analysis, was in agreement with Hallauer's (1978) summary of data. Ear length had 0% heritability due to a negative GCA variance and subsequently no additive variance associated with ear length (Table 3.17).GCA values, a reflection of additive effects, were significant (>.01) for tenderness, sweetness, DTS, DTA and RN (Table 3.19). PH and EH data produced GCA values significant at the 0.05 level, and GCA for ear length was not significant. SCA values were significant at the .01 level for sweetness and ear length, represented a significant non-additive effect associated with the expression of these traits (Table 3.19).

A high GCA/SCA ratio reflects strong additive effects and this was seen for both tenderness and row number, which also had the highest heritabilities of 74% and 55%, respectively (Table 3.20). The GCA variance and additive variance were also significant for tenderness and row number. Sweetness had a relatively high GCA/SCA ratio as well, but the SCA variance and non-additive variance were also more significant, close to 40% of the total variance, implying important non-additive effects. The error variances for

Table 3.19 Summary MS values from combining ability ANOV of data combined across locations for each trait.

	df	Tender	Sweet	DTS	DTA	PH	EH	RN	EL
GCA	8	***3.24****	***0.89***	***16***	***9***	**566****	**236**	***10***	0.51
SCA	36	***0.19***	***0.15***	5	3	251	77	0.74	0.74
ERROR	44	0.07	0.04	7	2	247	101	1.52	0.25

* *Bold italics indicate significance at .01*
** *Bold indicates significance at .05*

Table 3.20 Summary of GCA/SCA ratios and variance components from data combinded across all locations

TRAIT	GCA/SCA	Vgca*	Vsca	VA	VD	VE	VP
Sweet	5.82	0.07	0.11	0.13	0.11	0.04	0.29
Tender	16.93	0.28	0.12	0.55	0.12	0.07	0.75
Silking	2.98	1.00	0.00	2.01	0.00	7.24	7.57
Anthesis	2.71	0.56	1.36	1.12	1.36	2.24	4.73
Plant Height	2.25	28.64	3.92	57.27	3.92	247	309
Ear Height	3.05	14.45	0.00	28.90	0.00	101	106
Row Number	14.84	0.93	0.00	1.85	0.00	1.52	2.59
Ear Length	0.44	0.00	0.64	0.00	0.64	0.25	0.80

**Vgca = Variance associated with GCA*
Vsca = Variance associated with SCA
VA = Additive variance
VD = Non-additive variance
VE = Error variance
VP = Total variance

tenderness and sweetness were relatively low (14% and 9%), compared to the significant effect of location on tenderness and sweetness evaluations compared to PH, EH, and DTS.

3.4.3 Correlations of molecular marker data and phenotypic data

The SCA estimates represented the deviation of each cross from the array means in the diallel table of each trait. The r^2 and r values with SCA estimates were higher than those generated from the average phenotypic trait averages for all traits except tenderness and sweetness (Table 3.21). The correlations between genetic similarity values and both the average and the SCA estimates were all highly significant ($p \geq 0.01$) for DTS ($r = 0.72$), DTA ($r = 0.69$), PH ($r = -0.68$), and EH ($r = -0.51$) (Table 3.21). Only the SCA estimates of EL produced significant correlations with genetic similarities $r = -0.49$, ($p = 0.01$) and only the average values for tenderness significantly correlated with genetic similarity values $r = -0.32$ ($p = 0.05$). The sweetness and row number data were not significantly correlated with genetic similarities in this study (Table 3.21).

The traits of tenderness, sweetness, PH, EH, and EL all generated negative correlation coefficients with GS values. This implied that a high value of GS was correlated with a lower value of PH, EH, EL, and tenderness and sweetness ratings. This further illustrated that more similar inbreds showed less heterosis for those traits. The inverse was found with DTS and DTA, and implied that the more closely related inbreds showed increase in days to flowering (Table 3.21). RN correlations were positive, but low.

Table 3.21 Coefficients of determination (r^2) and correlation coefficients for genetic similarity values with average phenotypic values (AVG) across all traits and with SCA estimates (across all trials) associated with each trait and the respective significance level (p).

Trait	Coefficient of Determination (r^2)				Correlation Coefficient (r)			
	AVG	p	SCA	p	AVG	p	SCA	p
Tender	.10	.05	.04	ns	-.32	.05	-.20	ns
Sweet	.02	ns	.001	ns	-.13	ns	-.04	ns
DTS	.45	.001	.52	.001	.67	.001	.72	.001
DTA	.38	.001	.47	.001	.61	.001	.69	.001
PH	.47	.001	.48	.001	-.69	.001	-.68	.001
EH	.23	.01	.26	.01	-.48	.01	-.51	.01
RN	.006	ns	.01	ns	.08	ns	.10	ns
EL	.05	ns	.24	.01	-.23	ns	-.49	.01

**Bold and underlined indicates significance at .05*
*** Bold italics indicates significance at .001*
**** Bold indicates significance at .01*

3.5 Discussion

3.5.1 Molecular marker analysis and genetic diversity

Each of the 49 SSR markers used in this study to characterize the 10 tropical supersweet inbreds identified polymorphisms among the 10 inbreds. Molecular markers show a high level of polymorphism (Evola *et al.*, 1986), far exceeding that of isozyme markers (Stuber and Goodman 1983). There were high levels of polymorphism reported in other studies (91% to 100%) where a larger number of molecular marker loci were studied, 94 RFLP (Godshalk *et al.*, 1990), 164 RFLP (Melchinger *et al.*, 1990a) 82 RFLP (Livini *et al.*, 1992), 135 RFLP (Marsan *et al.*, 1998), and 70 SSR (Senior *et al.*, 1998).

There was high variation with lower marker number in this study that could be attributed to the diversity of the germplasm tested. In other related reports, primarily USA corn-belt field corns have been studied, that have a well-documented lack of genetic diversity (Hallauer, 1978). There is no reported diversity study utilizing molecular markers to evaluate genetic diversity in supersweet corns or primarily tropically derived inbreds. So-called exotic germplasm associated with the tropics is noted for its diversity (Hallauer, 1978). This was corroborated by a large survey of primarily mainland US field corn inbreds that found that most of the unique alleles were associated with tropical inbreds and pop corns that were included in the evaluation with SSRs (Senior *et al.*, 1998). Conversely, in this study the single temperate derived inbred, Ia453sh_2, had more unique alleles when compared with the tropical inbreds and was most distantly related to all other inbreds, verifying the intrinsic variation associated with tropical germplasm.

3.5.2 Phenotypic Trait Analysis

There was a significant location effect associated with DTS, DTA, PH, and EH in the combined analysis of these trails. This can be attributed largely to the Mealani location. The elevation (900m) and subsequently lower temperatures led to significant delays in flowering and reduction in the observed PH and EH. Although no temperature data was recorded, this effect was identified in the photoperiod study reported in Chapter 4 and showed that lower temperature significantly delayed flowering and reduced PH and EH. The large environmental effect could also explain the lower heritabilities generated for these traits-- DTS, DTA, PH, and EH-- compared with published findings (Hallauer and Miranda, 1981).

The location effect was not as dramatic for tenderness and sweetness indicating that these traits can be selected for equally well across locations. This was further enhanced by the significance of the GCAs of both of these traits ($F > .01$) and the high heritability and high additive variance associated with the expression of tenderness and sweetness (73% and 45%, respectively). This finding was in agreement with an earlier study that found a large GCA effect associated with kernel carbohydrate accumulation in sweet corn (Rosenbrook and Andrews, 1971). Carbohydrate level is considered to be the most significant component of perceived sweetness in sweet corn (Culpepper and Magoon, 1924). Rosenbrook and Andrews (1971) data was based on actual measurements of carbohydrate levels implying a correlation between actual carbohydrate measurement and the bite test used in this study.

The suitability of the bite-test method for tenderness evaluation has been previously shown to be a rapid, simple and effective evaluation method (Ito and

Brewbaker, 1981). This was verified in this study where simple correlations between trials was greater than 60%, and GCA, heritabilities and the additive variance were all high.

The sweetness and tenderness data also verified the usefulness of sib-pollinations within lines to reduce variability associated with the bite-test evaluations, as suggested by Ito (1983). A decrease of 6% and 5% in the variation associated with tenderness and sweetness bite-test evaluations was achieved through sib-pollinations within lines.

3.5.3 Correlations with hybrid performance

In other empirical studies on the relationship of grain yield performance of maize single crosses with RFLP distance between parental lines, correlations (r) of 0.09 (Godshalk, 1990), 0.14 (Dudley, 1991) , 0.32 (Lee, 1989) , and 0.46 (Melchinger, 1990) have been reported. The significant correlations between genetic similarity and DTS, DTA, PH, EH, and EL were equally or more significant than those previously reported, and the significant grain yield correlations reported by Marsan (1998) of r = 0.36 with RFLP and r = 0.51 with AFLP. The correlation of genetic similarity with tenderness was also significant, r = 0.32 at 0.05 level of significance in this study. The significant correlations in this study were achieved with a relatively low number of markers, (49).

The significant correlations between the genetic similarity values and some of the traits in this study suggest that these GS values would be suitable to predict hybrid performance of DTS, DTA, PH, EH, EL and to a lesser degree tenderness. Crosses using the inbred parent bA11 exemplify this GS by trait significance (Table 3.22). The inbred bA11 the most divergent inbred as compared to all others, with a GS value of 0.53 (Table

3.5). The cross bA11 x Kbtl13 had the lowest GS of all crosses, GS = 0.48. It would be expected that this cross would generate the most heterosis for DTS, DTA, PH, EH, EL and tenderness. The bA11 x Kbtl13 performed better than average for all traits except RN and EL where it performed equal to average (Table 3.22). It was not the sweetest cross, (ranking 5th out of 8). This reflected the failure of GS correlations to predict sweetness, as better correlations were found with tenderness evaluations and GS values. DTS, DTA, PH, and EH were all well above the average as predicted based on GS value. Overall, bA11 x Kbtl13 was the most interesting bA11 cross and exemplifies significant heterosis as would have been predicted based only on GS values.

Comparing performance of the crosses with 190, the most similar to all other inbreds, we see that higher GS values produce less superior hybrids (Table 3.23). The 217 x 190 hybrid with a GS value of .92 had a higher than average DTS and DTA and PH and EH were well below average. The 190 x 217 cross clearly shows a reduction in heterosis. Among the 190 crosses, the ba11x190 with the lowest GS value of .52 exhibited heterosis for the best correlated traits with a reduction in DTS and DTA and increase in PH and EH (Table 3.23).

Among the traits studied, tenderness, sweetness and row number were the most heritable, had the most significant GCAs, but were the least well correlated with molecular marker generated GS values. This could be attributed to the complexity of the traits and that the molecular markers used were not sufficiently diverse to include loci involved in the expression of the traits. Molecular markers are an arbitrary basis for analysis of heterosis as they are not necessarily correlated with the specific loci screened

Table 3.22 Summary of the genetic similarity values (GS), mean values for each trait in all bA11 crosses and the overall averages from all crosses, across all locations.

Hybrid	(GS)	Tender	Sweet	DTS	DTA	PH	EH	RN	EL
217 x bA11	(.52)	2.0	2.0	63	64	165	76	15	17
Hi36 x bA11	(.56)	3.2	3.5	61	62	185	84	18	17
Hi37 x bA11	(.53)	3.1	2.7	60	61	174	80	16	18
Hi38 x bA11	(.50)	3.1	1.8	62	62	161	71	14	18
bAll x bF47	(.58)	4.4	3.2	65	66	160	73	15	17
bA11 x Kbtl13	**(.48)**	**2.3**	**2.3**	**60**	**61**	**183**	**81**	**14**	**18**
bA11 x Kbtl32	(.54)	2.9	2.2	62	62	172	76	11	19
bA11 x 190	(.52)	2.5	2.2	62	63	174	87	15	17
Overall Averages		**2.8**	**2.5**	**64**	**63**	**155**	**68**	**14**	**18**

Table 3.23 Summary of the genetic similarity values(GS), mean values for each trait in all 190 crosses and the overall averages from all crosses, across all locations.

Hybrid	(GS)	Tender	Sweet	DTS	DTA	PH	EH	RN	EL
217 x 190	**(.92)**	**1.8**	**1.8**	**70**	**68**	**129**	**59**	**14**	**15**
Hi36 x 190	(.78)	1.8	2.3	65	64	154	78	17	19
Hi37 x 190	(.70)	2.0	2.4	65	64	152	74	16	19
Hi38 x 190	(.66)	2.4	1.9	66	64	151	65	14	18
bAll x 190	(.52)	2.5	2.2	62	63	174	87	15	17
Kbtl13 x 190	(.66)	2.0	1.5	63	62	167	74	13	19
Kbtl32 x 190	(.61)	2.5	2.4	64	63	154	64	13	19
Bf47 x 190	(.56)	3.5	2.5	65	63	149	68	13	18
Overall Averages		**2.3**	**2.1**	**65**	**64**	**154**	**71**	**14**	**18**

(Melchinger *et al.*, 1990b). Bernardo, (1992) reported that ideally at least 30-50% of the loci governing traits should be linked to molecular markers to allow useful screening. In addition, the trait should be highly heritable and have strong dominance effects to maximize prediction of hybrid performance based on molecular marker analysis.

This study illustrated that genetic similarity values generated from 49 SSR markers were sufficient in this germplasm to produce significant correlations, and were useful as a predictive tool for some of the traits. This would be enhanced by adding more SSR marker loci. This expectation is supported by grain yield study of Marsan (1998) who determined that AFLP markers produced more variants per loci than the RFLP markers used and higher correlations r = 0.51 (AFLP) than RFLPs (r = .36).

Specific gene locations for the traits in this study would enable us to make better choices about specific markers linked to those genes. This would be more informative and serve as a basis for selection and prediction of hybrid performance. This study further validated that molecular marker evaluation of inbred material was informative in determining genetic diversity and relationships among breeding materials (Lee *et al.*, 1989). The significance of correlations with genetic similarity and some of the traits studied in this research suggested that predictions of hybrid performance were attainable even with quality-associated traits like tenderness.

CHAPTER 4

IDENTIFICATION OF QTLs ASSOCIATED WITH PHOTOPERIOD RESPONSE IN A RECOMBINANT INBRED LINE POPULATION OF MAIZE

4.1 Abstract

Photoperiod sensitivity is a major reason that exotic tropical germplasm is not utilized in temperate breeding programs. This absence results in a limited germplasm base for temperate maize (Hallauer, 1978). One hundred RILs of the University of Hawaii Set G population derived from elite inbreds Hi31 (Hawaii/Iowa) and Ki14 (Thailand) and 10 parental sub-lines were evaluated in Mexico in 1997 and Hawaii in 1998 and 1999 for photoperiod response. Artificial lighting was used in all years to provide an extended photoperiod treatment of 17-17.5 hours. Traits measured were plant and ear height, days to silking and anthesis, and leaf number, all of which should be increased under and extended photoperiod in sensitive germplasm.

The 1997 and 1998 extended photoperiod treatments were effective in identifying Ki14 as the photoperiod sensitive parent as well as a sensitivity response in the RIL population. The days to anthesis, was extened from 25 and 27 days in Ki14 and 23 and 18 days in the RILs in 1997 and 1998, respectively, under extended photoperiod. Significant responses were measured in all traits under study in 1997 and 1998.

The Set G RIL population had been genotyped previously with 127 RFLP markers, creating a well-saturated linkage map (Ming *et al.*, 1997). Putative quantitative trait loci (QTLs) were analyzed through single-factor ANOVA and with MAPMAKER\QTL interval mapping. QTLs significantly associated with photoperiod response were found on chromosomes 1,2, 5, 7, 9, and 10. The QTLs with largest effect was associated with RFLP locus php06005 at 62 CM on Chromosome 10, and explained

from 9 to 39% of the variation within the population for the traits studied in both years, days to anthesis, days to silking, plant height, ear height, and leaf number below the ear. The finding of this region associated with QTLs based on the photoperiod response across traits measured and across years suggested this region was important to the genetic expression of photoperiod response in maize.

4.2 Introduction

As early as 1852 Henfrey concluded that the natural distribution of plants was at least partly due to latitudinal variations in summer daylengths (Thomas and Vince-Prue, 1997). Maize (Zea mays L.) is considered to be of tropical origin between the latitudes of 25 N and S. Due to man's migration and selection however, maize cultivation has been extended to latitudes beyond 50 N and S and is among one of the most widely adapted crop species (Duvick, 1996). The majority of current world production of maize is in temperate regions and less then four percent of temperate cultivars have any tropical or exotic germplasm in their pedigrees (Darrah and Zuber, 1985). Exotic germplasm comprises more than 250 races (Stevenson and Goodman, 1972) plus many additional tropically adapted and developed cultivars that represent a vast, untapped germplasm resource (Hallauer, 1978). Availability and the ability to utilize genetic diversity within a crop species are two major resources in any breeding program.

The genetic diversity in maize has not been fully exploited largely as a function of the variation in the adaptability to photoperiods at varying latitudes (Russell and Stuber, 1983a). Maize has been classified as a quantitative short day plant, with a rate of progress towards flowering declining in a linear fashion with increasing photoperiod

when that exceeding a critical period of 12-13 hours (Lee, 1978) (Bonhomme *et al.*, 1994). Tropical germplasm, is adapted to shorter photoperiods and has been shown to be more sensitive to changing photoperiods as they are moved north and south of the tropical zone (Brewbaker, 1981; Edmeades *et al.*, 1994; Ellis *et al.*, 1992b; Russell and Stuber, 1983a; Russell and Stuber, 1983b). This variation in adaptability consequently leads to a severe limitation in gene flow and effective exploitation of the diversity in maize by the plant breeder (Uhr and Goodman, 1995).

The photoperiod sensitive response is phenotypically materialized by variation in transition from the vegetative to reproductive phase of the plant leading to excessive vegetative growth and a delay of flowering. Traits which are subsequently evaluated to measure the photoperiod response include tassel initiation (Ellis *et al.*, 1992a; Russell and Stuber, 1983a), male and female flowering dates ((Bonhomme *et al.*, 1994; Edmeades *et al.*, 1992; Koester *et al.*, 1993), leaf number (Ellis *et al.*, 1992a; Russell and Stuber, 1983a), and plant and ear height (Khairallah *et al.*, 1998; Koester *et al.*, 1993; Schon *et al.*, 1994). Many studies have show that there is a genotypic component to photoperiod response in a variety of crops including maize. (Gu *et al.*, 1998; Lee, 1978; Logrono, 1990; Wallace *et al.*, 1993; White *et al.*, 1996). Lee (1978) showed through diallel analysis that photoperiod sensitivity is under polygenic control, and that associated traits of days to anthesis (DTA) and days to tassel initiation had high heritabilities. It has been noted that genetic conversion to low photoperiod sensitivity among tropical germplasm is relatively simple (Brewbaker, 1981) further supporting the high heritabilities of Lee's (1978) study.

Previous studies have identified QTLs in maize for traits associated with the some of the traits measured in this study, but not in association with photoperiod response. These traits include plant height, ear height, days to silking and anthesis, and leaf number (Beavis et al., 1991; Khairallah et al., 1998; Koester et al., 1993; Ragot et al., 1995; Ribaut et al., 1996; Schon et al., 1994; Stuber et al., 1992). One study of near isogenic lines, NILs, identified a maturity QTL on chromosome eight and is speculated to be specifically associated with photoperiod response (Koester et al., 1993). Screening germplasm for photoperiod requires elaborate field, greenhouse or phytotron experiments with artificial light sources (Bonhomme et al., 1994; Lee, 1978; Logrono, 1990; Russell and Stuber, 1983a) or multi-locational screening (Bonhomme et al., 1991) which require extensive logistical planning and expense. The identification of molecular markers tightly linked to QTLs or genes of major effect with the photoperiod response would allow for marker assisted selection (Van Berloo and Stam, 1998) during the backcross conversion process as has been utilized for the photoperiod sensitivity trait in rice (Mackill et al., 1993). This would allow the plant breeder to develop adapted materials with increased genetic variability in a shorter time without the expense and time consuming field screening.

Understanding the genetic basis of photoperiod sensitivity has potential uses in other applications, aside from marker assisted selection. In the case of pearl millet, the manipulation of photoperiod sensitive alleles with non-sensitive genotypes has allowed the modification of flowering dates in pearl millet inbred lines improving the synchrony of flowering for hybrid seed production schemes (Bidinger *et al.*, 1999).

The objective of these studies was to (1) evaluate the traits associated with photoperiod response; days to silking and anthesis, leaf number, and plant and ear height in the Set G maize RIL population both under normal and artificially extended photoperiods, (2) to identify QTLs associated with the expression of the traits associated with photoperiod response, and (3) to elucidate more about the genetic control of traits associated with photoperiod sensitivity in maize.

4.3 Materials and Methods

4.3.1 Plant Material

The maize population in these studies was Hawaii Set G, a recombinant inbred line (RIL) population based on the hybrid of inbreds Hi31 and Ki14. The temperate dent line Hi31 was derived from the inbred lines B68 and RB14A out of 'Iowa Stiff Stalk Synthetic', and the tropical flint line Ki14 was derived from 'Suwan 1', a Thai flint population (Brewbaker, 1997). The F1 plants were self-pollinated and carried through to F7 via single seed descent, resulting in 129 RILs (Moon and Brewbaker, 1999). This population was previously genotyped with 127 RFLP probes, and a well saturated linkage map was developed of total length 1625 cM, with an average interval of 15.8 cM between markers (Ming *et al.*, 1997). Twenty sub-lines of each parent were bred and evaluated as sister lines to provide greater accuracy in the assessment of parental line means and variances.

4.3.2 Field Evaluations

The Set G RILs and parents were evaluated in paired field trials. One utilized a control planting under normal photoperiod of approximately 12 hours (NOR) and second planting under an extended photoperiod of 17.5 hours (EXT). One set of trials was planted at the CIMMYT research station in Tlaltizipan, Mexico in 1997, and the others at the University of Hawaii Research Farm in Waimanalo, Hawaii in 1998 and 1999.

The 1997 field evaluations were conducted at the experimental station of the International Maize and Wheat Improvement Center (CIMMYT) near Tlaltizipán, Mexico, the site was located at 940 m altitude in the central mountains of Mexico (18°41'N, 99°08'W). Three replications of 100 Set G RILs plus 10 sub-lines of each parent were planted on December 17, 1997 in 1.5m plots with 2 seeds every 15cm and thinned to one plant per hill in a field with solid-set lighting system (Figure 4.1). The lights were shut off between 9 pm and 3:30 am each day from January 5, 1997 until tasseling to provide a 17.5 hour photoperiod. The control planting also included 3 replications planted on December 17, 1997, in 2.5m plots with 2 seeds every 20cm and later thinned to one. Due to a shortage of field space 2 replications were planted at one location and the third replication planted at another location on the station.

The 1998 trial was planted at the Waimanalo Research station of the University of Hawaii on Oahu at 20m altitude and 21°N, 158°W. The control planting (March 10,1998) of two replications of 100 Set G RILs and 10 sub-lines of each parent were planted in 2.5m plots. The extended day treatment of the same germplasm was planted under lights on March 19, 1998. The extended day treatment utilized artificial light from 150-watt

Figure 4.1 Solid set lighting system used at the Tlaltizipan Research Station of CIMMYT.

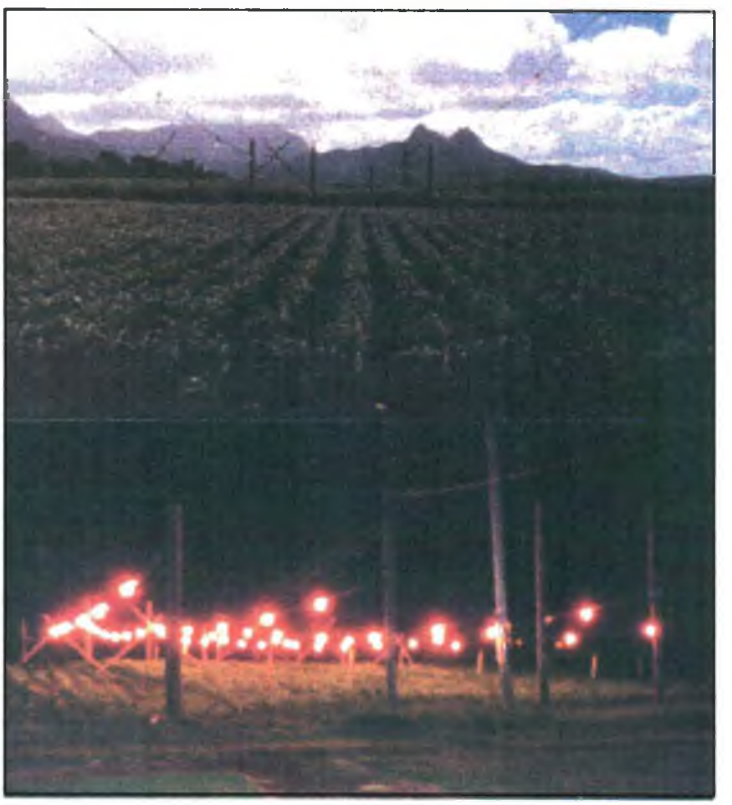
Bulbs which were strung on 51m lines with 10 bulbs on each and approximately 4.6m between lines. There were additional tripod supports at approximately 25m in the 51m row (Figure 4.2). This lighting apparatus was based on earlier work at Waimanalo by Lee (1978) and Logrono (1990). Lights for the extended day period were turned on from 6pm until 11pm each evening with an automatic timer, extending the photoperiod to approximately 17 hours.

The 1999 trial was also planted at the Waimanalo Research Station, with 2 replications of the same germplasm and with the previously described artificial light source. The control treatment was planted on May 25, 1999 and the extended day treatment was planted on April 1, 1999. Lighting on this trial initially failed and was operational only after about May 10, 1999.

Data collected for both treatments of all trials included

- Days to 50% anthesis (DTA) and days to 50% silking (DTS)
- Plant height (PH) on 6-8 plants per line measured from base of the plant to the uppermost node
- Ear height (EH) on 6-8 plants per line measured from the base of the plant to the base of the uppermost ear
- Total leaf number and leaf number above the ear data were collected in 1997 and leaf number below the ear (LN(be)) values were derived by subtraction. Leaf number below the ear (LN(be)) data were collected in 1998, but no leaf number data were collected on the 1999 trial.

Figure 4.2 Lighting system used at the Waimanalo Research Station in 1997 and 1998. (Photo from **(Logrono, 1990)**)



4.3.3 Statistical Analysis

ANOVAs for all traits (PH, EH, DTS, DTA, LN(be)) in both treatments (EXT and NOR) for the years 1997 and 1998 were computed individually and combined across years according to the spreadsheet method of Brewbaker (1996). The program MAPMAKER/QTL (Lincoln *et al.*, 1993) was used to identify putative QTLs associated with all traits measured in association with photoperiod sensitivity. This included the data on PH, EH, DTS, DTA, LN(be) for both the normal and extended photoperiod treatments. The differences in the treatments were also analyzed with MAPMAKER/QTL. The data are presented with LOD scores that represent the log of the odds ratio between the probability that the data would arise from a QTL with this effect divided by the probability that it would arise due to chance alone (Paterson, et al., 1988). All of the data were also analyzed using the GLM procedure of SAS (SAS Institute, 1989) to determine the association between RFLP loci and the traits associated with photoperiod sensitivity.

4.4 Results

4.4.1 Field Evaluations

The DIFF values for Ki14 are significantly higher for all traits than those values for Hi31 (Table 4.1), firmly establishing Ki14 was the more photoperiod sensitive parent, as expected based on the tropical background of this inbred. DTA and DTS were extended about one month more in the parents and RILs in Tlaltizipan in 1997 than in the 1998 Waimanalo trial. This difference could be attributed to temperature differences. No temperature data was taken, records for Tlaltizipan (1999) and Waimanalo (1970) show that the average temperatures differ only by about 2° C, but the night temperatures in

Table 4.1 Parental average values in two years for five traits and ranges and averages for the RIL population.

Trait	Year	Hi31			Ki14			RILs				
		NOR**	EXT	DIFF	NOR	EXT	DIFF	NOR	NOR	EXT	EXT	DIFF
		avg	avg	avg	avg	avg	avg	range	avg	range	avg	Avg
DTA*	1997	90	98	8	92	117	25	50-110	88	90-120	111	23
	1998	63	79	16	69	96	27	45-80	67	70-100	85	18
DTS	1997	92	107	15	95	120	25	50-104	91	82-125	113	22
	1998	63	81	18	69	101	32	45-75	67	70-115	91	24
PH	1997	130	128	-2	133	155	22	95-175	128	95-195	142	14
	1998	173	187	14	157	212	55	120-205	164	118-240	190	26
EH	1997	66	72	6	73	110	37	22-105	69	39-130	85	16
	1998	74	75	1	82	144	62	45-118	76	20-160	89	13
LN(be)	1997	13	14	1	15	22	7	12--16	14	13-22	17	3
	1998	12	12	0	14	17	3	11--15	13	11--17	13	0

* *DTA = days to 50% anthesis*
DTS = days to 50% silking
PH = plant height
EH = ear height
LN(be) = leaf number below the ear
***NOR = normal photoperiod treatment*
EXT = extended photoperiod treatment
DIFF = difference between the normal and extended response

Tlaltizipan are much cooler and could explain the significant delay in flowering observed in 1997 (Table 4.2). It was similarly observed by Jong (1980) in Hawaii observed that DTS was decreased 4.29 days per °C (Jong, 1980). There was also an average increase in PH and EH of 45cm and 11cm, respectively, in the 1998 trial (Table 4.1) due also to temperature difference.

The difference between years and LN(be) was not clear (Table 4.1), but this could be attributed to the variation in the manner that the leaf number data was collected in the two trials. In 1997 total leaf number values and leaf numbers above the ear were counted on each plant per line. This was initiated at the five leaf stage and the 5^{th} and 10^{th} leaves were cut so that this could be noted at the time that counts were recorded. At least the first five leaves would have fallen off by the time of flowering. From these data, leaf number below the ear was extrapolated and it was this trait that showed the most variation between the lines in 1997. In 1998 only leaf numbers below the ear were counted on 6 plants per line at one time after flowering. The data consequently represents an under-estimation of leaf number. The 1998 leaf count data was adjusted by adding 7 to each value, but this only puts the numbers within the same range and did not necessarily reflect the total effect on LN(be). The difference between the parents was still discernable as well as the slight effect on the range of the RILs for LN(be), but the overall mean of the RILs was no different in 1998 (Table 4.1).

In comparing the 1999 results (Table 4.3) it was clear that the differences between the parental averages were not as great for all traits measured as the 1997 and 1998 data. DTA for Ki14, for example, showed a photoperiod response of 25 and 27 days and Hi31 a response of 8 and 16 days in 1997 and 1998, respectively. In 1999 (Table 4.3) the DTA

Table 4.2. Temperatures in °C for Tlaltizipan and Waimanalo for first three months after plantings were made.

Location	Month	Maximum	Average	Minimum
Tlatizipan (1999)	January	35	19	5
	February	34	21	7
	March	37	23	9
Waimanalo (Lee 1970)	March	25	22	18
	April	26	23	19
	May	27	24	21

Table 4.3. Average values in 1999 for each trait for parents and ranges and averages for the RIL population in each year for every trait.

TRAIT	Hi31			Ki14			RILs				
	NOR	EXT	DIFF	NOR	EXT	DIFF	NOR	NOR	EXT	EXT	DIFF
*DTA	56	63	7	59	69	**10**	45-70	56	50-70	64	**8**
DTA	60	64	**4**	61	71	**10**	52-64	57	59-77	66	**9**
PH	204	196	**-8**	186	192	**6**	146-243	194	152-244	188	**-6**
EH	102	100	**-2**	117	118	**1**	76-155	110	66-150	101	**-9**

difference is only 10 days for Ki14 and 7 days for Hi31. This finding was further demonstrated by the RIL population results. In 1999 the RIL averages for DTA only differed by 8 days while in 1997 and 1998 they differed by 23 and 18 days respectively.

The RIL population distributions under normal (RILSMFDno) and extended (RILSMFDex) photoperiods for DTA in all three years showed that in the 1997 and 1998 distributions there was a clear separation between the two treatments reflecting the photoperiod response (Figure 4.3). The 1999 distribution was not as significant, illustrating the lesser degree of variation generated in that trial. The shift in the distributions of the RILs under the normal photoperiod treatment (upper distribution) there was little difference between the parental distributions (Figure 4.4, 4.5). In the extended photoperiod distribution there was a clear separation of the parental sub-lines with Ki14 showing a significant photoperiod response in both years (Figure 4.4,4.5).

The same RIL population and parental distributions for the 1999 trial indicated a slight effect on the RILs and Ki14 (Figure 4.6), but not as was significant as in 1997 and 1998. This response was corroborated for all other traits measured (DTS, PH, and EH) in the 1999 trial. It was clear that the artificial extended photoperiod treatment was not effective in producing the photoperiod response. This was due to problems with setting up the lighting system and lack of monitoring of the system to ensure it was functioning properly.

Figure 4.3. Distribution of days to anthesis for RIL populations under normal and extended photoperiod treatments in 1997, 1998, and 1999.

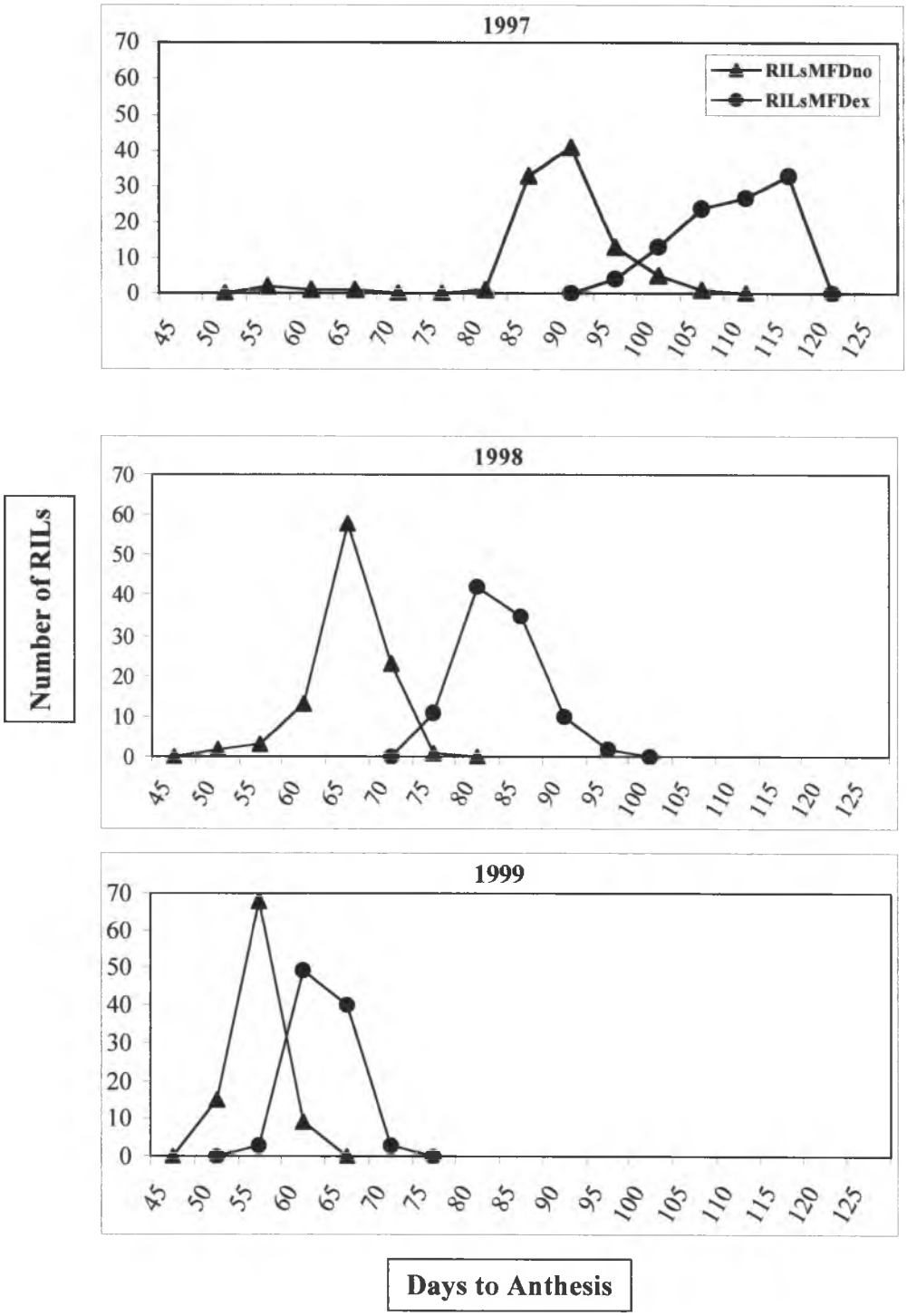


Figure 4.4. 1997 Distributions of parents and RILs for days to anthesis under normal and extended photoperiod treatments.

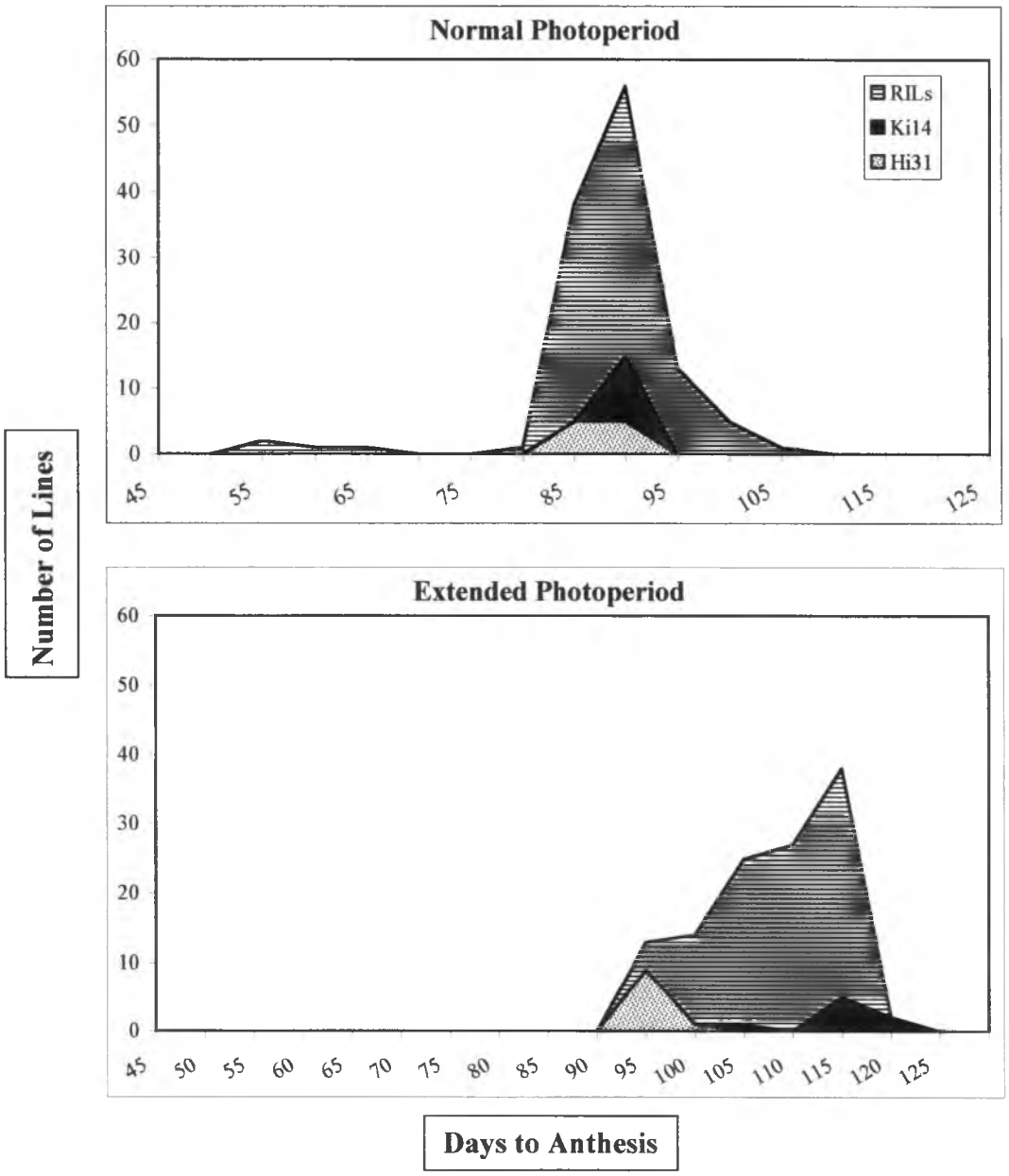


Figure 4.5. 1998 Distributions of parents and RILs for days to anthesis under normal and extended photoperiod treatments.

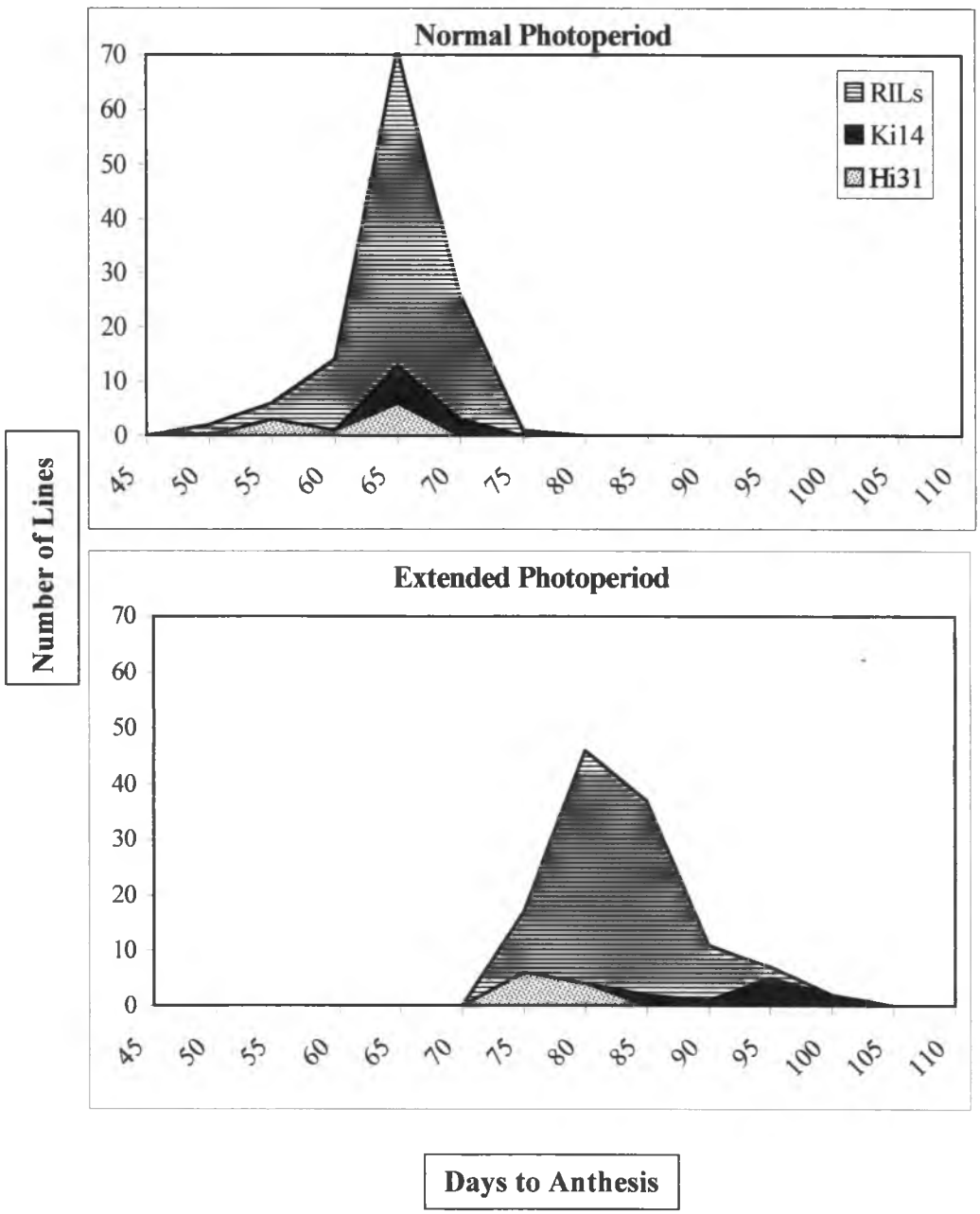
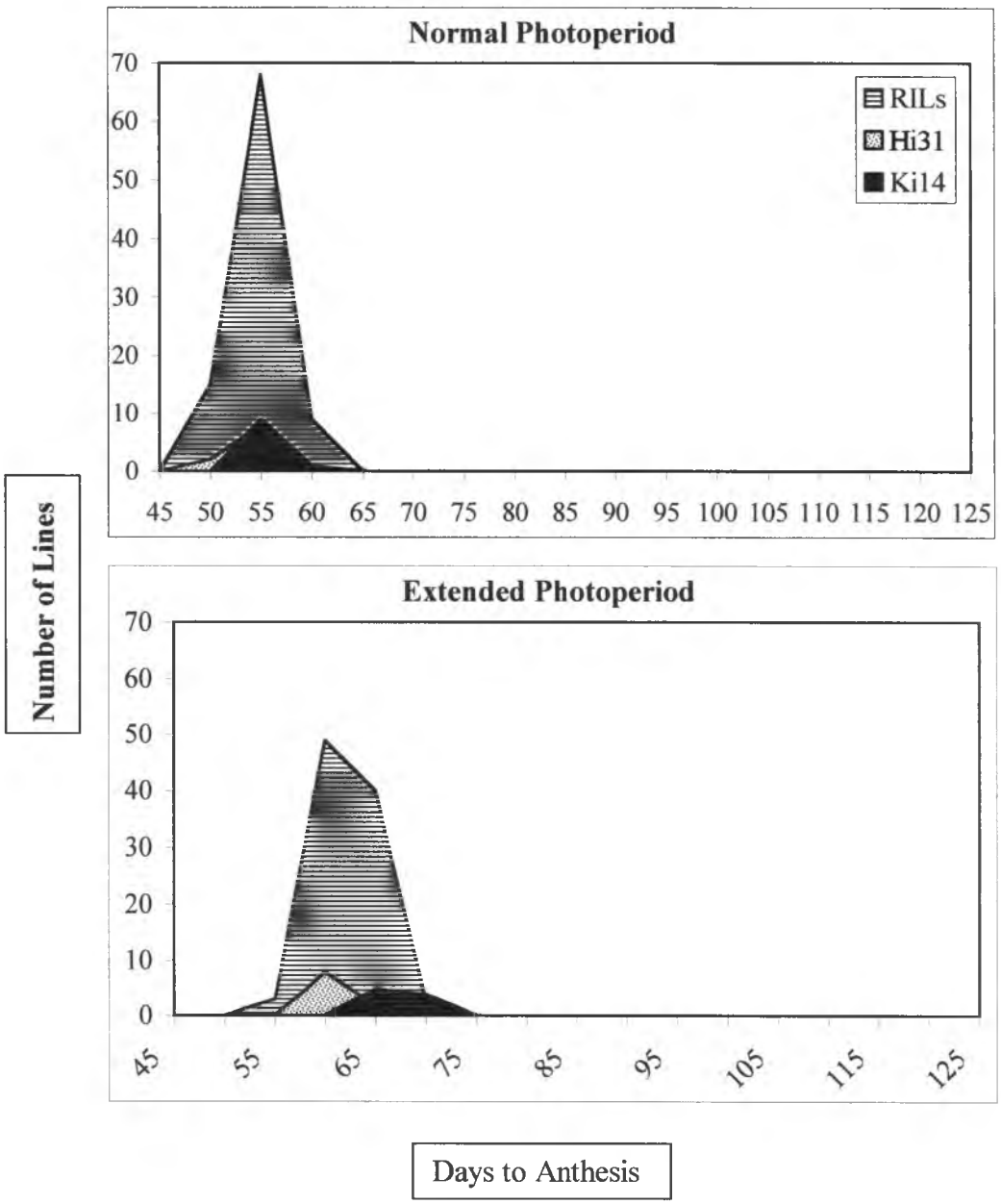


Figure 4.6. 1999 Distribution of parents and RILs for days to anthesis under normal and extended photoperiod treatments.



4.4.2 QTL Analysis

QTL analysis was performed on data for all five traits under both normal and extended photoperiod, as well as the differences between the treatments in both 1997 and 1998. MAPMAKER/QTL was used with a LOD score threshold of 2.0 for each experiment. Intervals significantly associated with one or more traits occurred on all chromosomes except chromosome 6. Single factor analysis of variance performed with the GLM procedure of SAS revealed 13 RFLP markers on chromosomes 1,2,5,7,8,9, and 10 associated with intervals that accounted for 8% or more of the variation associated with the phenotypic expression of the traits.

QTLs on chromsomes 1,2,5,7,8, and 9 that were significant in either or both the 1997 and 1998 seasons were identified (Table 4.4). Treatment' refers to the normal photoperiod (normal) or extended photoperiod (extended) treatments or the difference between them (difference). The specific RFLP marker and chromosome (CHR) associated with the QTL were identified as well as the LOD score from MAPMAKER/QTL analysis and the percent of the variation in the population that the locus explained (VAR%) based on the SAS analysis.

The QTL for PH on chromosome one associated with php20855 at 168 cM in bin 1.09 was only identified from the 1998 data. The closest reported QTL for plant height was between the interval umc83 - csu164 in bin 1.10. It was noted that there are more than 27 loci associated with plant height (Sheridan, 1988). It was not surprising that of the other putative QTL identified in association with PH on chromosomes 5,7 and 8 had no confirmation based on previous findings.

Table 4.4 Putative QTL identified on chromsomes1,2,5,7,8, and 9 by MAPMAKER/QTL and confirmed by the GLM procedure of SAS for traits and treatments in 1997 and 1998.

			1997			1998		
TRAIT	TREATMENT	CHR	marker	LOD	%VAR	marker	LOD	%VAR
PH	normal	1				php20855		11
DTA	extend	2	umc5	3.62	12	umc5	3.34	12
DTA	difference	2	umc53	3.46	17		3.4	
EH	extend	2				npi287	2.43	10
EH	normal	2				npi287	3.29	12
LN(be)	normal	2				npi287	3.23	9
PH	normal	5				bnl 5.71	2.41	11
PH	extend	5				bnl 5.71	3.32	8
EH	normal	5				bnl 5.71	2.91	11
EH	extend	5				bnl 5.71	2.9	11
PH	difference	7	umc110	3.67	10			
PH	normal	8	umc173b	4.14	13			
DTA	difference	9				bnl7.50	2.72	20
DTA	extended	9	umc81	2.17	18			
LN(be)	difference	9	umc81	4.35	14			
LN(be)	extended	9	umc81	3.27	8			

The putative QTLs identified on chromosome 9 associated with umc81 (50cM) for DTA difference in 1997 and LN(be) difference and extended in 1997 were of interest as Alber (1992) found association with DTS at *acp1* locus that is mapped at 51 cM. Although no association with DTS at this locus was found in this study, the findings at this location suggested that it may be important in the expression of flowering and warrants further analysis. Overall the QTLs on chromosomes 1,2,5,7,8 and 9 were significant, but only DTA extended treatment data identified QTLs on chromosome 2 associated with umc5 in both years.

The RFLP marker php06005 at 64cM on chromosome 10 showed association with each of the measured traits DTA, DTS, PH, EH, and LN(be) (Table 4.5). The RFLP locus php06005 in association with DTA extended photoperiod data in 1997 and 1998 had LOD score values of 4.17 and 2.63 and explained 30 and 10% of the variation within the population. A similar result was generated in the analysis of the 1998 DTA difference data with a LOD score of 4.17 and explaining 12% of the variation. Similarly, the PH and EH difference data from both 1997 and 1998 produced QTLs attributed to this locus (Table 4.5).

The extended photoperiod data of PH in 1998 and EH and 1997 also identified this locus as explaining 15 and 19% of the variation, respectively. Both the DTS and LN(be) difference data produced significant evidence of a significant QTL in 1998 and 1997, respectively (Table 4.5). Overall, LN(be) difference data from 1997 with a LOD score of 9.07 explained 39% of the variation, and was the most significant QTL identified.

Table 4.5 Putative QTL identified on chromosome 10 by MAPMAKER/QTL and confirmed by the GLM procedure of SAS for the traits and treatments in 1997 and 1998.

CHROMOSOME 10							
		1997			1998		
TRAIT	TREATMENT	Marker	LOD	%VAR	Marker	LOD	% VAR
DTA*	difference**				php06005	7.09	12
DTA	extend	**php06005**	**4.17**	**30**	**umc44**	**2.63**	**10**
DTS	difference				php06005	6.04	25
PH	difference	**php06005**	**4.18**	**20**	**php06005**	**4.19**	**9**
PH	extend				php06005	4.02	15
EH	difference	**php06005**	**7.13**	**31**	**php06005**	**9.75**	**13**
EH	extend	php06005	4.38	19			
LNBE	difference	php06005	9.07	39			

**DTA = days to anthesis*

DTS = days to silking

PH = plant height (cm)

EH = ear height (cm)

LN(be) = leaf number below ear

***difference = differences between the normal and extended photoperiod response used in QTL analysis*

extend = values for the extended photoperiod treatment used in QTL analysis

The results of the MAPMAKER/QTL analysis for chromosome 10 were graphed for DTA, DTS, LN(be), PH, EH, respectively (Figures 4.7 to 4.11). In all scans the x-axis represents the intervals along chromosome 10 and the y-axis values the respective LOD score values produced at that location. The extended photoperiod data for DTA from both years produced very similar profiles (Figure 4.7), while the 1997 DTA difference data resulted in a more significant QTL in the same region. Single highly significant QTLs around the php06005 locus for DTS and LN(be) in 1998 and 1997, respectively (Figure 4.8, 4.9). The QTLs identified in association with plant height in this same region were visualized are all comparable (Figure 4.10).

The scans generated from the EH data differences in 1997 (EH97) and the extended photoperiod treatment in 1997 (ExtEH97) represent the other QTLs identified with relatively gently sloping scan profiles (Figure 4.11). The profile for EH differences in 1998 (EH98) however, was an example of a possible false positive generated from MAPMAKER/QTL due to the dramatic peak. The expectation for a valid QTL was reflected by a gradual increase in the LOD score values associated with the region of interest that reached a peak and declined in a similar gradual fashion. The sharp peak like the one in Figure 4.11 illustrates that perhaps the degree of this QTL was overestimated by MAPMAKER/QTL as there was a gradual rise on either side of the divergence from that. This was further confirmed by the SAS results that indicate that 13% of the variation associated with EH differences in 1998 was explained by the php06005 as compared to 30% with the EH difference data in 1997. The confirmation of the importance of the locus with SAS analysis in association with the 1998 difference data suggested that

Figure 4.7. Mapmaker scan generated on chromosome 10 for days to anthesis differences data in 1997 (DTA97) and extended photoperiod data in 1997(ExtDTA97) and 1998 (ExtDTA98)

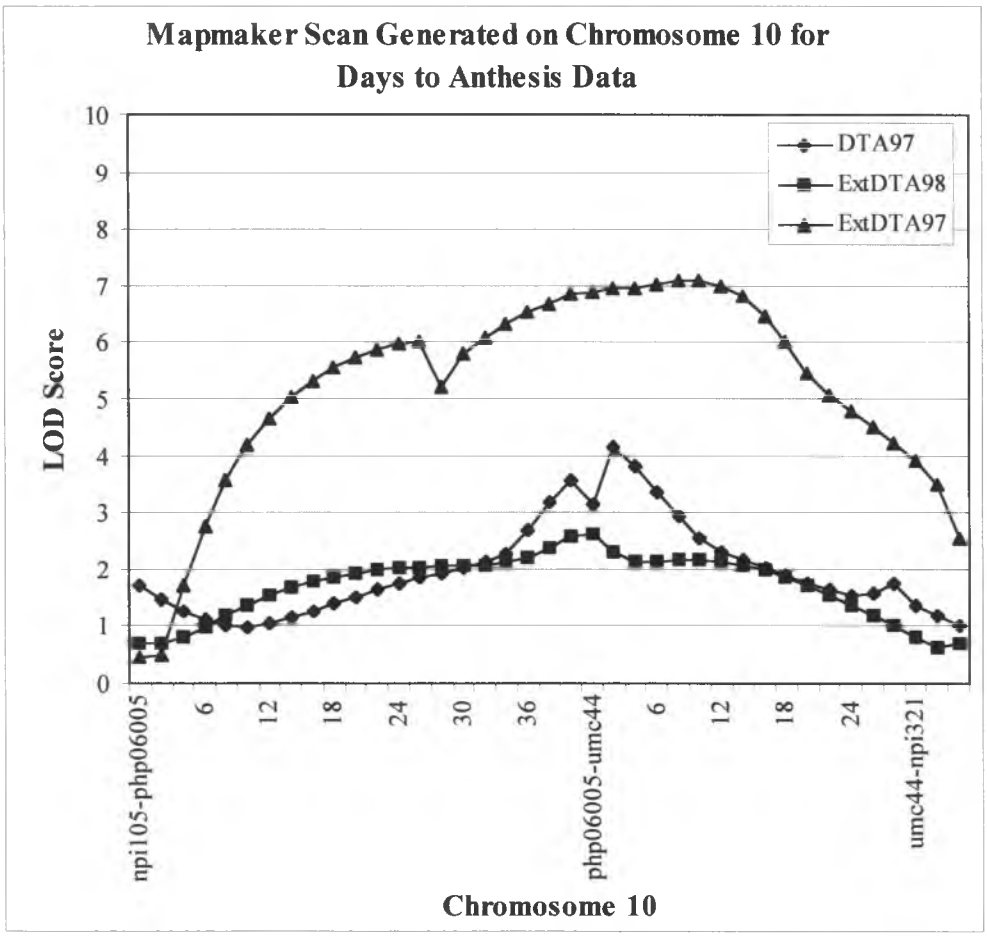


Figure 4.8. Mapmaker scan generated on chromosome 10 for days to silking differences data in 1998 (DTS98).

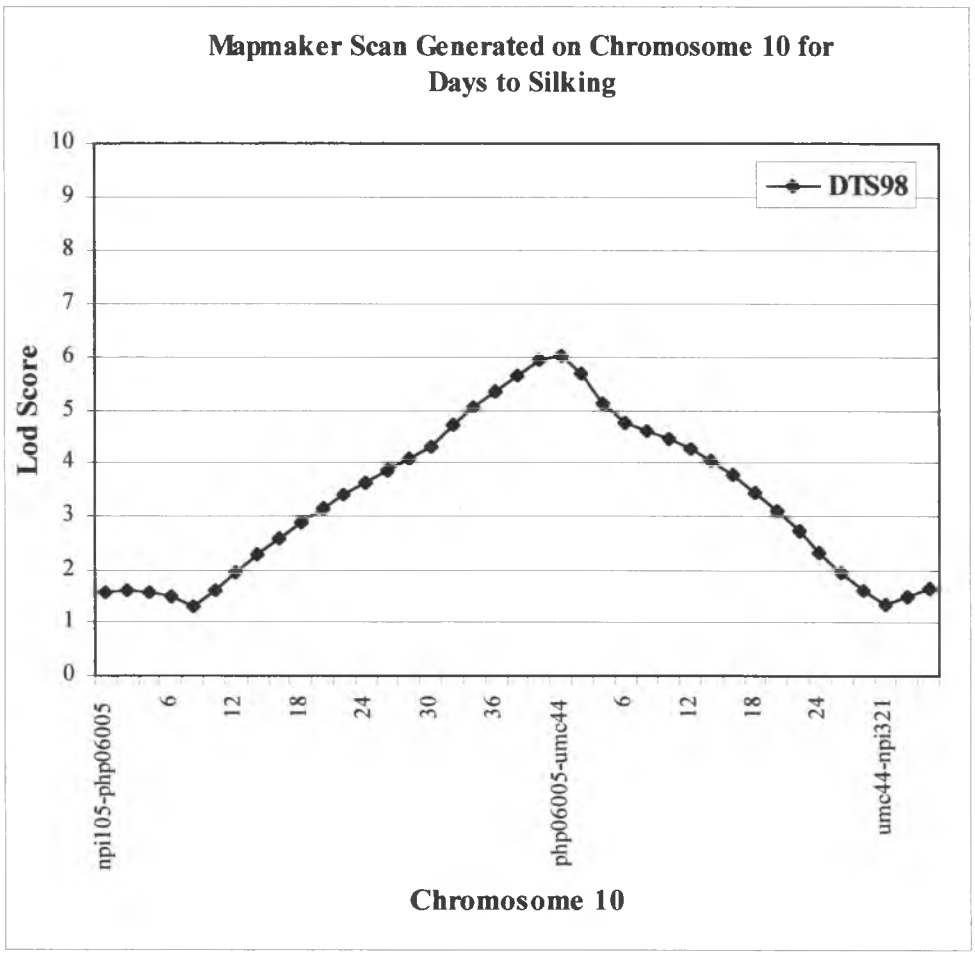


Figure 4.9. Mapmaker scan generated on chromosome 10 for leaf number below the ear differences in 1997 (LN(be)97).

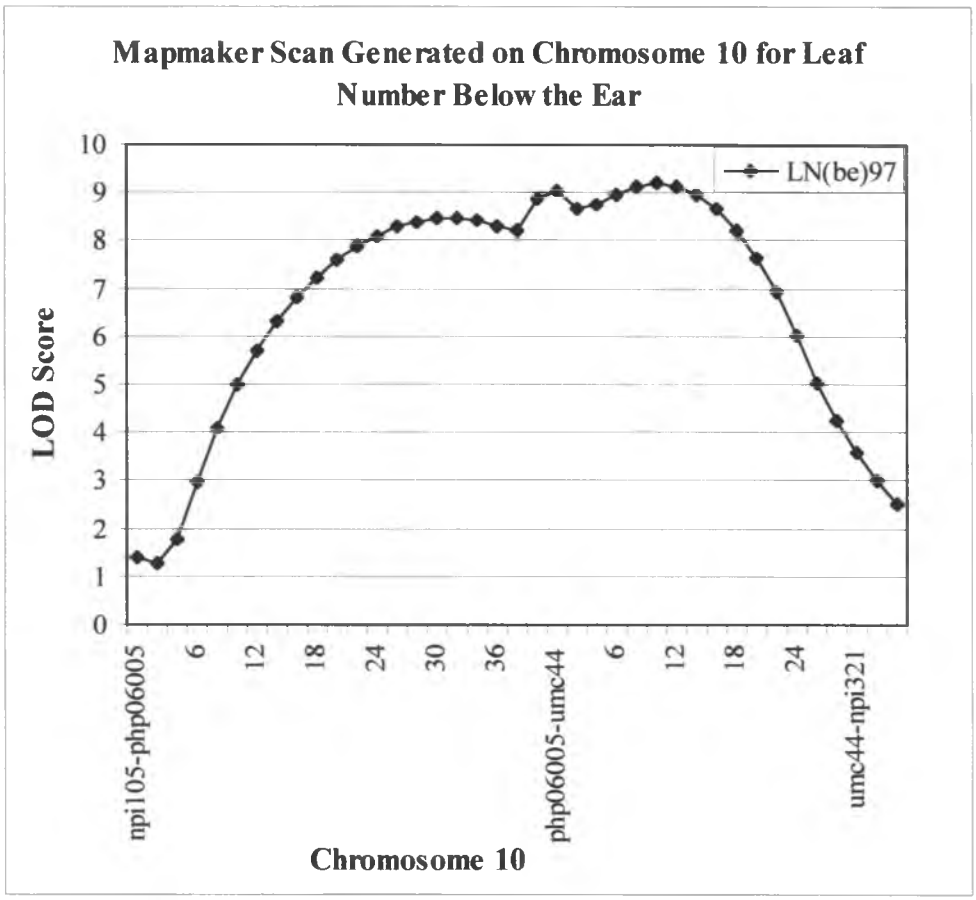


Figure 4.10. Mapmaker scan generated on chromosome 10 for plant height differences data in 1997 (PH97) and 1998 (PH98) and extended photoperiod data in 1998 (ExtPH98)

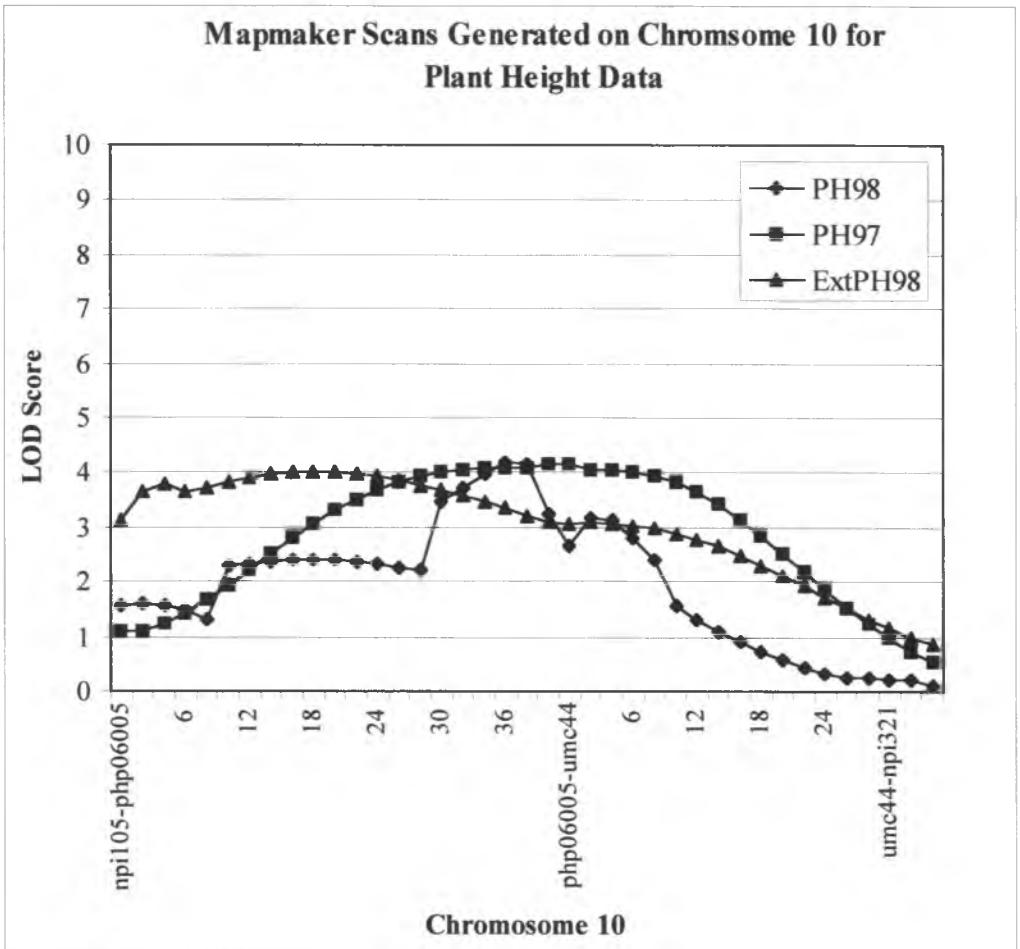
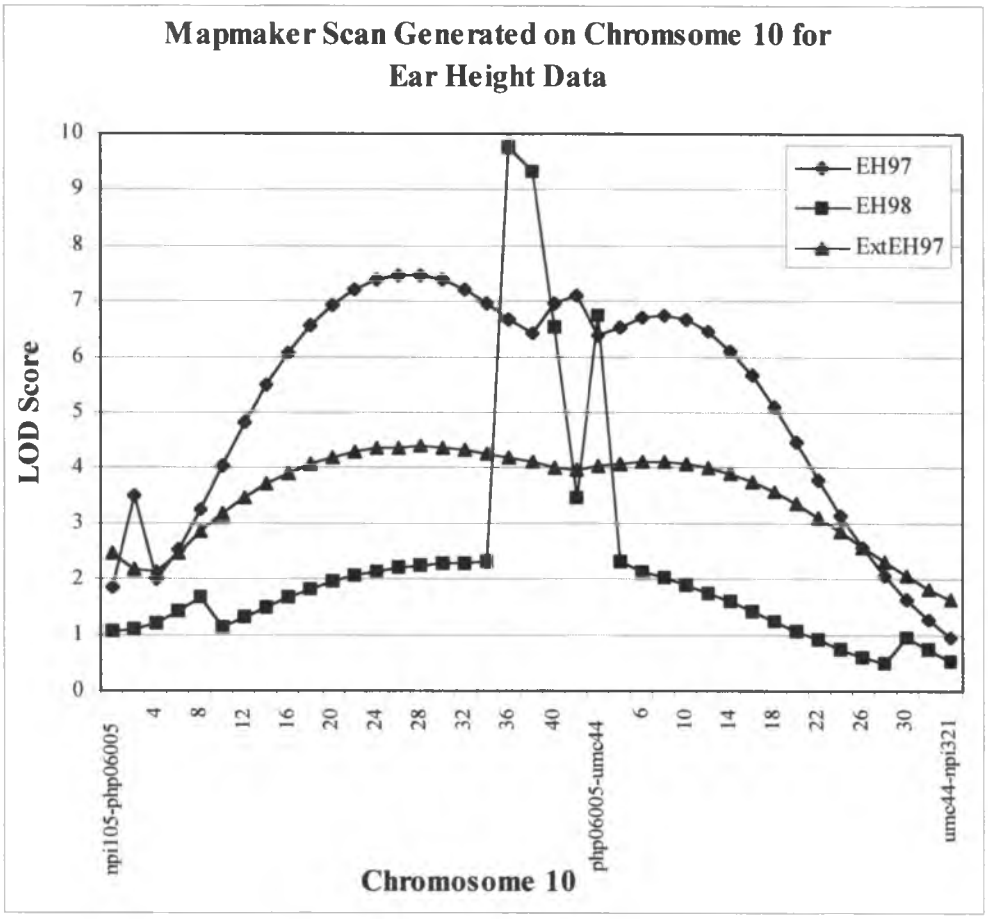


Figure 4.11. Mapmaker scans generated on chromosome 10 for ear height differences data in 1997 (EH97) and 1998 (EH98) and extended photoperiod data in 1997 (ExtEH97)



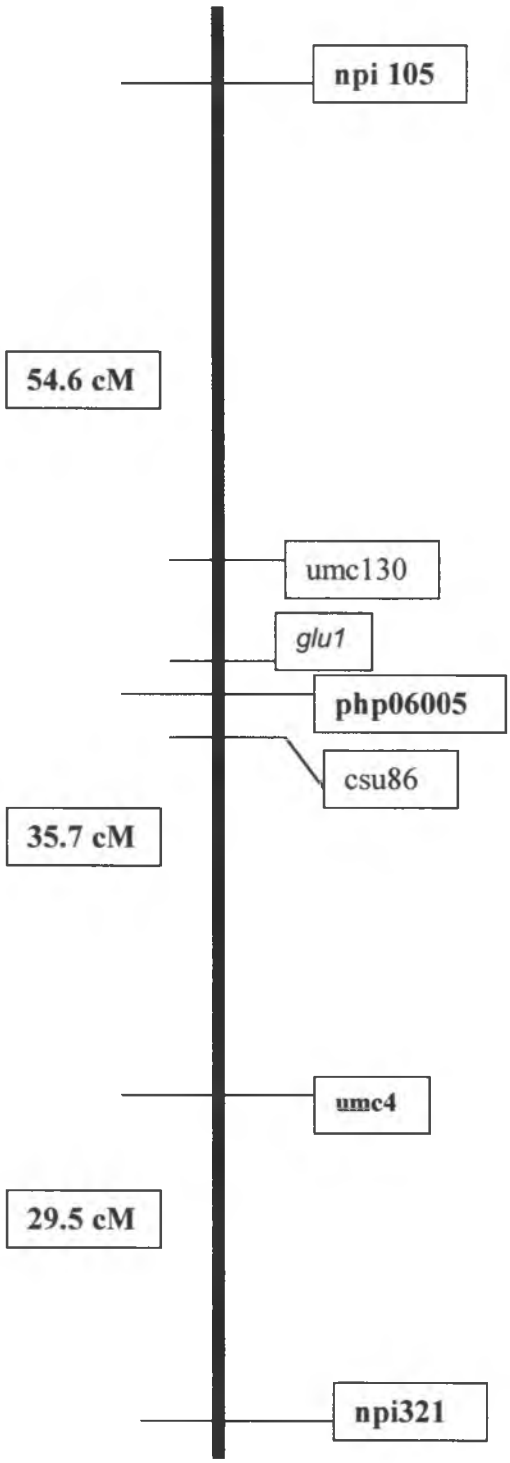
MAPMAKER/QTL overestimated the maximum LOD values, but that there was still probably a real QTL associated with this marker and the 1998 EH difference data.

4.5 Discussion

The RFLP marker php06005 at 64 cM on chromosome 10 showed association with all of the traits that were measured to evaluate photoperiod response in this study. The confirmation across years in the case of PH, EH and DTA indicates an even stronger relationship of this region with the expression of photoperiod sensitivity. That only the extended daylength treatments and the photoperiod effect, or the difference in the treatments, were associated with php06005 further demonstrates the importance of this region to photoperiod response. This finding at this region was supported by the study of Alber *et al.* 1991 (Alber *et al.*, 1991) who identified an association of the isozyme effect on plant height near *glu1* (Figure 4.12). This was no direct correlation between the marker *glu1* (62 cM) and days to silking. Stuber *et al.* 1992 also identified a large relationship to photoperiod sensitivity, however it suggests that this region has also demonstrated an effect on these traits associated with the expression of photoperiod sensitivity.

Other reported correlations with this region on chromosome 10 include a QTL for anthesis silking interval associated with csu86 at 67cM (Ribaut *et al.*, 1996). This is not a major QTL, and has a LOD score of 2.40 and explains 5.8% of the variation. This is in close proximity however, to the QTLs identified in this study and it is suggested by Bonhomme *et al.* 1994 that photoperiod response is positively correlated with anthesis

Figure 4.12 Representation of chromosome 10 with Set G markers (bold) and other markers associated with the findings in this study.



silking interval (R^2=.62). Another QTL associated with DTA was found by Koester et al. (1993) at umc130 at 52cM which explains 6.8 and 2.5% of the variation associated with DTA in two populations of near isogenic lines, NILs, that they studied (Figure 4.12). This further confirmed the importance for this location being important to the expression of the traits associated with photoperiod response.

Koester (1993) suggested a region on the short arm of chromosome 8 also associated with photoperiod response. That region was not confirmed in this study, but other studies on the genetic basis of photoperiod sensitivity suggest a number of genes may be responsible for the expression of this trait in diverse germplasm. More than a single locus has been associated with photoperiod response in all quantitative genetic and breeding studies (Lee, 1978). One study looking at maturity estimated from four to six effective factors (Koester *et al.*, 1993) and a study of silking delay estimated 1-14 genes under extended photoperiod (Logrono, 1990). PH expression alone is believed to have association with more than 27 loci (Sheridan, 1988).

The degree of phenotypic effect on PH, DTA, DTS in this study were in agreement with those found in the earlier study of Koester (1993). In the Koester study, they evaluated germplasm under naturally occurring photoperiods with a cold winter season in Florida and a hot summer in North Carolina. The similarity of response between studies further validates the utility of the artificial lighting system for evaluation of photoperiod sensitivity.

The coverage of chromosome 10 with 4 RFLP markers was limited with the Set G linkage map. Increased saturation of the region with more molecular markers would provide confirmation and increased clarification of the importance of the region on the

photoperiod sensitivity response. The Set G population has been increased from 100 to 230 RILs and study of the new RILs should also further enhance these initial findings. The fixed nature of RIL populations also allows for an extension of studies beyond the current test locations, and unlimited environmental possibilities for evaluation of photoperiod response and confirmation of these findings.

CHAPTER 5

SUMMARY

5.1 Lime-Induced Chlorosis

A major QTL associated with lime-induced chlorosis in maize was identified in this research on the long arm of Chromosome 3. This corroborates the translocation study of Champoux et al. (1988) that identified the long arms of Chromosomes 3 and 8 as sites of major genes for tolerance. The present study further showed that the RIL segregations of SPAD readings for chlorosis were closely approximated by a statistical model invoking two major QTLs for tolerance.

The major QTL on chromosome 3 associated with lime-induced chlorosis might be at or near the chromosome 3 location of the yellow stripe mutant of maize, *ys*3. This mutant is thought to affect the plant's capacity to secrete phytosiderophores, leading to interveinal chlorosis under Fe-limiting conditions (Basso *et al.*, 1994). Our study did not identify any QTL on Chromosome 8, but putative minor QTLs occurred on Chromosomes 2, 4, 5, 7, and 9. The study by Champoux et al. (1988) used visual ratings of chlorosis and stunting of plant height as indices, and identified also regions on Chromosomes 2, 4, 6 and 10 as affecting tolerance (Champoux *et al.*, 1988).

5.2 SSR Analysis and Hybrid Performance Prediction of Sweet Corn Inbreds

Each of the 49 SSR markers used in this study to characterize the 10 tropical supersweet inbreds identified polymorphisms among the 10 inbreds. The high variation with lower marker number in this study that could be attributed to the diversity of the germplasm tested. In other related reports, primarily USA corn-belt field corns have been

studied, that have a well-documented lack of genetic diversity (Hallauer, 1978). There is no other reported diversity study utilizing molecular markers to evaluate genetic diversity in supersweet corns or primarily tropically derived inbreds. So-called exotic germplasm associated with the tropics is noted for its diversity (Hallauer, 1978). This was corroborated by a large survey of primarily mainland US field corn inbreds that found that most of the unique alleles were associated with tropical inbreds and pop corns that were included in the evaluation with SSRs (Senior *et al.*, 1998). Conversely, in this study the single temperate derived inbred, Ia453sh_2, had more unique alleles when compared with the tropical inbreds and was most distantly related to all other inbreds, verifying the intrinsic variation associated with tropical germplasm.

In field evaluations the location effect was not as dramatic for tenderness and sweetness evaluations indicating that these traits can be selected for equally well across locations. This was further enhanced by the significance of the GCAs of both of these traits ($F > .01$) and the high heritability and high additive variance associated with the expression of tenderness and sweetness (73% and 45%, respectively). This finding was in agreement with an earlier study that found a large GCA effect associated with kernel carbohydrate accumulation in sweet corn (Rosenbrook and Andrews, 1971). Carbohydrate level is considered to be the most significant component of perceived sweetness in sweet corn (Culpepper and Magoon, 1924). Rosenbrook and Andrews (1971) data was based on actual measurements of carbohydrate levels implying a correlation between actual carbohydrate measurement and the bite test used in this study.

This research verified the suitability of the bite-test method for tenderness evaluation which has been previously shown to be a rapid, simple and effective

evaluation method (Ito and Brewbaker, 1981). In this research simple correlations of tenderness evaluations between trials was greater than 60%. The GCA values, heritabilities and the additive variance were all high as well.

The sweetness and tenderness data also verified the usefulness of sib-pollinations within lines to reduce variability associated with the bite-test evaluations, as suggested by Ito (1983). A decrease of 6% and 5% in the variation associated with tenderness and sweetness bite-test evaluations was achieved through sib-pollinations within lines.

The significant correlations between genetic similarity and DTS, DTA, PH, EH, and EL were equally or more significant than those previously reported, and the significant grain yield correlations reported by Marsan (1998) of $r = 0.36$ with RFLP and $r = 0.51$ with AFLP. The correlation of genetic similarity with tenderness was also significant, $r = 0.32$ at the 0.05 level of significance in this study.

This study further validated that molecular marker evaluation of inbred material was informative in determining genetic diversity and relationships among breeding materials (Lee *et al.*, 1989). The significant correlations in this study were achieved with a relatively low number of markers, (49) and suggest that the GS values generated would be suitable to predict hybrid performance of DTS, DTA, PH, EH, EL and to a lesser degree tenderness.

5.3 Photoperiod Response

QTLs significantly associated with photoperiod response were found on chromosomes 1, 2, 5, 7, 9, and 10 in this research. The QTLs with largest effect were associated with RFLP locus php06005 at 62 CM on Chromosome 10, and explained from

9 to 39% of the variation within the population for the traits studied in both years, days to anthesis, days to silking, plant height, ear height, and leaf number below the ear. The finding of this region associated with QTLs based on the photoperiod response across traits measured and across years suggested this region was important to the genetic expression of photoperiod response in maize.

The degree of phenotypic effect on PH, DTA, DTS in this study were in agreement with those found in the earlier study of Koester (1993). In the Koester study, they evaluated germplasm under naturally occurring photoperiods with a cold winter season in Florida and a hot summer in North Carolina. The similarity of response between studies further validates the utility of the artificial lighting system for evaluation of photoperiod sensitivity.

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